

The Australian Wine Research Institute



Annual Report

2000

Council Members

Mr D.J. McWilliam, BSc, MSc
Chairman—Elected a member under Clause 6(e) of the Articles of Association

Mr R.E. Day, BAgSc, BAppSc
Elected a member under Clause 6(e) of the Articles of Association

Mr R.L. Gibson, BAppSc, BAppSc
Elected a member under Clause 6(e) of the Articles of Association

Mr P.F. Hayes, BSc, BAppSc, MSc, DipEd
Elected a member under Clause 6(e) of the Articles of Association

Professor P.B. Høj, MSc, PhD
Ex officio under Clause 6(d) of the Articles of Association as Director of the Institute

Mr T.W.B. James, AssDip(WineProd)
Elected a member under Clause 6(e) of the Articles of Association

Mr G.R. Linton, BAppSc(AppChem), GradDip(SysAnal)
Elected a member under Clause 6(e) of the Articles of Association

Professor G.R. Scollary, MSc, PhD, BEd, BAppSc(Wine Science), FRACI
Charles Sturt University Representative under Clause 6(c) of the Articles of Association

Professor M. Sedgley, PhD
The University of Adelaide Representative under Clause 6(b) of the Articles of Association

Dr R.R. Walker, BAgSc(Hons) PhD
CSIRO Representative under Clause 6(a) of the Articles of Association

Registered office
Waite Road, Urrbrae, SA 5064

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The Company

The Australian Wine Research Institute was incorporated under the South Australian Companies Act on 27 April 1955. It is a company limited by guarantee, it does not have a share capital and it has been permitted, under licence, to omit the word 'limited' from its registered name.

The Memorandum of Association of The Australian Wine Research Institute sets out in broad terms the aims of the Institute and the Report of the Committee of Review for the Institute published in March 1977 identified the following specific aims:

1. To carry out applied research in the field of oenology.
2. To service the extension needs of the winemakers of Australia.
3. To be involved in the teaching of oenology at both undergraduate and postgraduate levels.
4. To assume responsibility for the co-ordination of oenological activities, and the collection, collation and dissemination of information on oenological and viticultural research to the benefit of the Australian wine industry.

The Institute's laboratories and offices are located on the Waite Campus of The University of Adelaide at Urrbrae in the Adelaide foothills, on land leased from the University. The original lease is for a term of 99 years, with a right of renewal clause for a further 99 years. The Institute formally affiliated with The University of Adelaide in 1990. The first buildings were erected and opened in 1957 and alterations and extensions were completed in 1976. The buildings have been extensively modified and refurbished since that time with major extensions being undertaken in 1994 and 1999.

The Institute is adjacent to the Faculty of Agricultural and Natural Resource Sciences of The University of Adelaide, three Divisions of the CSIRO and the South Australian Research and Development Institute.

Table of Contents

2	Chairman's report
3	Council notes
4	Director's report
6	Staff
7	Highlights of the year
8	Staff activities
10	Scientific Research Teams' Reports
32	Industry Services Teams' Reports
48	Abridged financial report
52	Appendix 1-5

46th Annual Report
30th June 2000

Presented to the Australian
Wine Industry

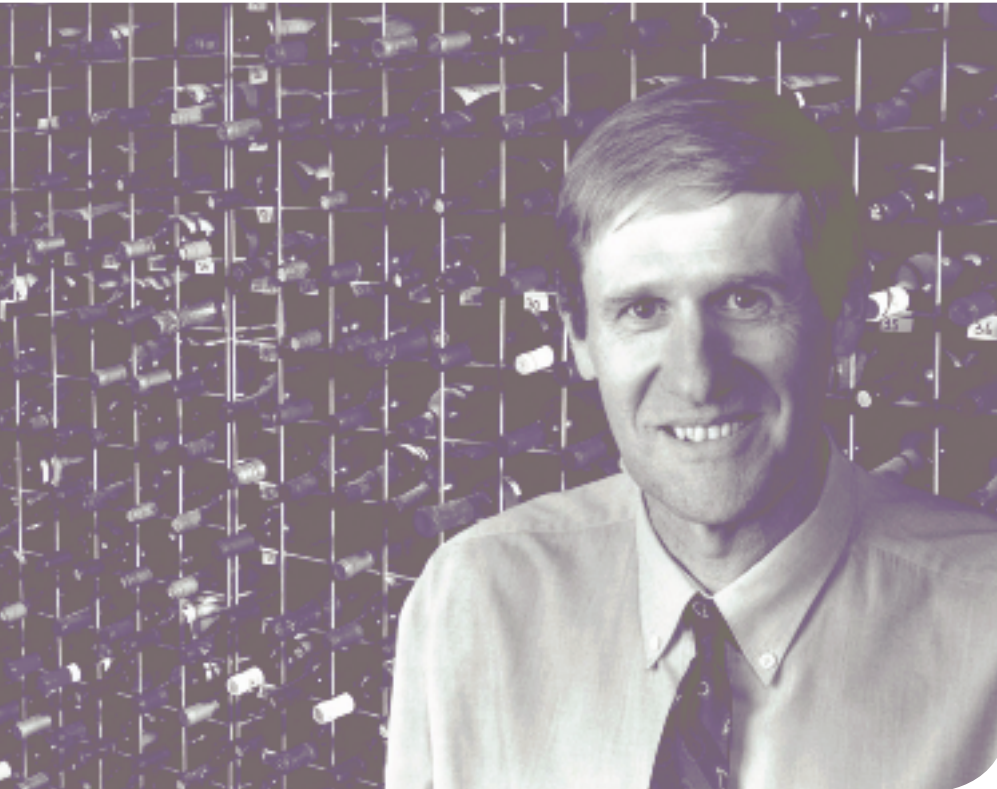


Annual Report
2000

The Australian Wine Research Institute



Chairman's report



Doug McWilliam, Chairman of Council

It is with great pleasure that I present the 1999-2000 annual report for The Australian Wine Research Institute. The most obvious change to all who visit, is the new building extensions that include an expanded Analytical Service laboratory, staff room, meeting room and offices. This wonderful extension of our facilities was completed during the year and the Institute was delighted to have Barbara Hardy AO open the new building on 26 October 1999. Barbara has given great support to both the Institute and our industry over many years and generously supports the production of our Institute's bi-monthly publication, *Technical Review*.

The new building was completed on budget and apart from housing expanded Institute facilities also provides a home for the Australian Society for Viticulture and Oenology who now have an office in the new facility. Our newly expanded Analytical Service has installed the latest GC-MS equipment dedicated to grape pesticide and fungicide residue analysis, TCA analyses and oak analyses, these being areas where the Institute sees itself as being able to provide a very specialised service of increasing demand.

The Analytical Service welcomes Don Buick

as its new leader and Don's challenge will be to bring further automation and sophistication to the services offered. These services, I am pleased to say, have increased further by about 15% this year and has now exceeded the one million dollar per year milestone. The surplus from this Service supports Australian wine research and now gives our Institute a degree of independence when it comes to capital expansion and replacement rather than being totally committed to industry levy funds.

I wish to thank the GWRDC for its continued confidence in our Institute particularly for the advanced contributions to the new building and also for equipping the Waite Campus Mass Spectrometry Facility with a new research grade TSQ GC-MS. Council's invitation to have a GWRDC Director sit in on all regular AWRI council meetings has also been accepted. This has allowed the greatest scrutiny of our industry-funded activities, of which we have no fear. It is also pleasing to note that the GWRDC is prepared to negotiate longer term funding for core projects. This, hopefully, will put us on a multi-year, rather than annual, cycle of funding similar, in principle, to that currently offered to the

CRCV II. This will add much needed continuity to major programs and a degree of staff security which has become a significant issue when attempting to procure and employ experienced and well qualified people from the highly competitive industry such as we have.

The Co-operative Research Centre for Viticulture II is now well underway and the Institute's Principal Research Biochemist, Dr Elizabeth Waters, is leader of Program 1, titled *Vineyard management to meet grape quality specifications*. The Institute's major involvement is in a number of projects where significant progress has been made, for instance the 'Tannin Wheel' produced in collaboration with Adelaide University, and the use of near infrared spectroscopy as a potential means of measuring grape quality parameters which has been run in collaboration with industry partners.

The Industry Services team continues to provide assistance and advice to winemakers. The recently established and progressing 'closure trial' has attracted great attention, not only from our own winemakers but also from research establishments overseas. It is hoped that the results obtained from this work will further refine the testing protocols for new types of closures as they become available to industry.

Finally, I wish to welcome Peter Hayes as a new member of Council and we are pleased that his presence will strengthen the viticultural interests and expertise of our board. I must also thank our retiring Councillor, Geoff Weaver, who over the past eight years provided valuable guidance and help to all members of our Institute. I offer a special thanks to our Director, Peter Høj, his team leaders and all of his staff for the high standard of their contributions throughout the past year. Their combined endeavours have ensured, as I stated last year, that our Institute remains the envy of the rest of the oenological world.

Doug McWilliam
Chairman of Council



Council notes

Chairman

At the Council Meeting held on 29 February 2000, Mr D.J. McWilliam was re-elected Chairman of Council.

Members of the Executive Committee

Mr D.J. McWilliam
Mr R.L. Gibson
Mr P.F. Hayes
Professor P.B. Høj
Mr T.W.B. James
Professor M. Sedgley

Deputy Members of Council

Mr L.P. Deans
Dr P.R. Dry
Mr J. Northey
Dr N.S. Scott
Dr A.M. Smith
Mr P.J. Wall

Meetings

Ordinary General Meeting
The 45th Ordinary (Annual) General Meeting was held on 26 October 1999.

Council

The Council of the Institute met on the following dates: 27 July 1999, 26 October 1999, 29 February 2000 and 2 May 2000. Executive members of Council met on 30 November 1999.

Funding

The Council of the Institute acknowledges the continuing financial support of the Grape and Wine Research and Development Corporation and the Cooperative Research Centre for Viticulture.

Appreciation

The Institute acknowledges the assistance and cooperation of the following organizations throughout the year:

Adelaide University
Australian Wine and Brandy Corporation
Charles Sturt University
Commonwealth Scientific and Industrial Research Organization (CSIRO)
Cooperative Research Centre for Viticulture
Department of Agriculture, Forestry and Fisheries Australia
South Australian Wine and Brandy Industry Association
State Departments of Agriculture
Winegrape Growers' Council of Australia, Incorporated
Winemakers' Federation of Australia Incorporated

Left to right: Mr T.W.B. James, Professor P.B. Høj, Dr P.R. Dry (Deputy for Professor M. Sedgley), Professor G.R. Scollary, Mr D.J. McWilliam, Dr R.R. Walker, Mr P.F. Hayes, Mr G.R. Linton, Mr R.L. Gibson and Mr R.E. Day





Director's report



Peter Høj

The knowledge economy and the Australian wine industry

It is said increasingly that maintaining and further developing a prosperous society is dependent on individuals, companies, institutions and industries embracing the knowledge economy. Said more simply, in a world with declining tariff barriers, economies which are not characterised by a high degree of innovation will not remain competitive and will increasingly lose market share. There is a general consensus that the Australian society is good at making discoveries but comparatively poor at turning such discoveries into innovations of high export or import replacement value. Symptomatic of this situation is Australia's previously relatively high Government expenditure on R&D and its very low and declining Business

Expenditure on R&D (BERD, which more appropriately should be named Business Investment on R&D or BIRD). The reasons for the low BERD in Australia, now one of the lowest in the OECD, are not easy to pinpoint but they may well be related to the wealth of natural resources in Australia, combined with an economy which, for large parts of the twentieth century, was protected by tariff barriers acting as a buffer against more innovative overseas competitors. The combined consequences of these two scenarios would be that a business culture in which investment in innovation is seen as a natural prerequisite for prosperity has not been developed adequately and that the drivers to establish fruitful collaborations between publicly funded research organisations and business have not been optimal.

Like many other industries, the wine industry invests relatively little cash in R&D and yet the industry is generally regarded as having received much of its current success through innovation. These two issues are not always mutually exclusive. Whilst it is true that the industry invests too little cash in R&D, it does invest significant human capital in the R&D game. Large sectors of the Australian wine industry have arguably succeeded where many other Australian enterprises are still lacking, namely establishing a culture committed to acquisition and application of new knowledge. Specifically, in submitting its bold vision for annual sales of \$4.5 billion by 2025 in the Strategy 2025 document, the Australian wine industry adopted the mission statement: 'Total commitment to innovation and style from vine to palate.' A main driver for this goal is a commitment to increased research and development, and to further develop the existing wine industry learning culture. These commitments, in turn, define three critical elements for industry innovation:

1. *an enhanced commitment to research and development through increased financial contributions and industry participation;*
2. *an enhanced effort to secure technology diffusion through communication of research issues to industry; and*
3. *an enhanced ability of the work force to receive and critically evaluate technical issues of increasing sophistication.*

Without appropriate research funding, new knowledge is not generated and, without continual industry input, the right research will not be performed. Without communication, even the most useful research and development outcomes remain meaningless. Finally, without an educated work force, even the best research and communication strategy is inconsequential. For innovation to be truly ingrained in an industry, the elements of R&D, technology diffusion and staff education must come together in a seamless fashion. The industry led Institute, through its stated aims and core activities, is helping to achieve this and the response rate from industry is heartening. Increasingly, our projects are strengthened by direct involvement of industry practitioners. Recent examples include: the industry reference group advising on design and conduct of our wine closures

trial; the industry reference group advising on our tannin research; and several wineries committing their precious resources to assist with evaluation of new yeasts. Over and above that, we now see a willingness from industry to invest in R&D additional to what can be derived from the research levies. The current activity in the area of Near Infrared Spectroscopy is a clear example of this. Through additional direct investment and in-kind contributions, we have been able to progress development of affordable rapid instrumentation techniques at an accelerated pace and we have been pleased to see that the outcomes of this research are already starting to be implemented in industry. The willingness of companies to commit staff and cash to such joint developments point further to the unique pre-competitive sharing of resources in the wine industry and also to the fact that the industry is further strengthening its innovation culture, so prevalent in many successful overseas companies. We welcome this development and work to accommodate the issues that, notwithstanding the spirit of pre-competitive sharing of efforts, will flow from differential investment in R&D through the Institute. The Institute is aware of its obligations to all levy payers in Australia and is committed to finding workable solutions to new issues as they arise. Meanwhile we will enjoy the increasing commitment to R&D from a large part of our industry as it clearly is a

prerequisite for future success in the knowledge economy. In the global economy, we will only remain competitive with an enhanced R&D investment in areas which turn opportunity into revenue and prosperity.

An important aspect of our activities is to tap into the knowledge base in other institutions and companies worldwide. The Institute has, therefore, chiefly through funding from two different Cooperative Research Centers, recently established firm links with researchers at Flinders University, the University of Melbourne and INRA at Montpellier. We do so in the belief that, managed appropriately, there is more to be gained from tapping into global knowledge than to be lost. The close relationship between industry and wine/grape researchers in Australia makes it overwhelmingly likely that our industry will transform research outcomes into practice at a greater rate than overseas competitors. A recent example of this philosophy is the appointment of a postdoctoral fellow, Dr Stéphane Vidal, to a joint position at INRA Montpellier and at the Institute. At Montpellier, Dr Vidal will be supervised by Dr Veronique Cheynier, arguably one of the foremost researchers on wine tannins in the world, and, at the Institute, Dr Vidal will form part of our tannin group supervised by Dr Liz Waters. The complementarity of Dr Cheynier's skills in tannin chemistry and of the Institute's strong knowledge of wine and its sensory evaluation, creates a win-win

situation from which both parties will benefit. The extent to which various wine industries will benefit depends on the efficiency of knowledge transfer and implementation.

As mentioned by our Chairman, key Institute staff have during the past year again contributed magnificently in their efforts to meet output targets and to service industry in the best possible way and I thank them for that. A special thanks goes to our former Viticulturist, Alex Sas. Alex has, after four most productive years with the Institute, taken up a position as Central Viticulturist with BRL Hardy and we wish him the best of luck. The diffusion of staff back into the industry is a particularly pleasing occurrence. It is to be hoped that building of human capital in the wine industry will be further enhanced by increased recruitment of personnel which have received tertiary training in an institution which emphasises the importance of closing the gap between the science and its application.

Peter Høj
Director



Staff

Peter Bordier Høj, MSc, PhD, Copenhagen, Director

Research team members

Paul Anthony Henschke, BSc(Hons) PhD *UAdel.*, Principal Research Microbiologist

Mark Aidan Sefton, BSc(Hons) PhD *UWA*, Principal Research Chemist

Elizabeth Joy Waters, BSc PhD *UAdel.*, Principal Research Biochemist

Ian Leigh Francis, BSc(Hons) *Monash*, PhD *UAdel.*, Senior Research Chemist

Yoji Hayasaka, DipEng(IndChem) Tokyo *I.T.*, MPharm *Vic. Col. Pharm.*,

CertIntBusMgt *Monash*, Manager—Mass Spectrometry Facility

Miguel Antonio de Barros Lopes, BSc *Oregon*, PhD *UC (Santa Barbara)*, Molecular Biologist

Eveline Jutta Charlotte Bartowsky, BSc(Hons) PhD *UAdel.*, Research Microbiologist

Zhong Kui Peng, BSc MSc, *Zhejiang*, PhD *UAdel.*, Research Chemist

George Kyriakos Skouroumounis, BSc(Hons), *Flinders*, PhD *UAdel.*, Postdoctoral Research Fellow

Gordon Michael Elsey, BSc(Hons), PhD, *Flinders*, Postdoctoral Research Fellow (commenced 13/12/99)

Michael Brian Esler, BSc(Hons) *USydney*, PhD *UWollongong*, Postdoctoral Research Fellow (commenced 28/2/00)

Stéphane Vidal, Eng Dip. (Biochem), *Nat. Instit. App. Sc. Lyon*, MBiochem, *UClaude Bernard Lyon*, PhD *UJoseph Fourier Grenoble*, Postdoctoral Research Fellow (commenced 5/6/00)

Kenneth Frank Pocock, BAppSc *UAdel.*, FAIFST, Senior Chemist

Alan Percy Pollnitz, BSc(Hons) *UAdel.*, Senior Chemist

Daniel Sejer Pederson, BSc, MSc, *Copenhagen*, Visiting Research Chemist (concluded duties 28/2/00)

Michael Raunkjaer, MSc, *Copenhagen*, Visiting Research Chemist (commenced 1/3/00)

Peter James Costello, BSc (Hons) MSc *UNSW*, PhD *UAdel.*, Microbiologist

Holger Gockowiak, BSc(Hons) *UAdel.*, Microbiologist

Jeffrey Mark Eglinton, BSc(Hons) *UAdel.*, Microbiologist/Computer Systems Officer

Josephine Newton, BSc, PhD, *UAdel.*, Casual Research Chemist (maternity leave from 1/6/00)

Stella Kassara, BSc(Hons) *UAdel.*, Chemist (maternity leave from 30/6/00)

Anthony John Heinrich, BBiotech(Hons) *Flinders*, Research Assistant

Dimitra Capone, AssDip(Chem) *USA*, Laboratory Technician (10 months' leave from 25/2/00)

Maria Jolanta Kwiatkowski, MSc *Gliwice*, Laboratory Technician

Wieslawa Cynkar, BSc PhD *Wroclaw*, Technical Officer

Jenny Bellon, Part-time Laboratory Assistant

Shauna Liam Brown, BBiotech(Hons) *Flinders*, Postgraduate Student

Anita Oberholster, BSc(Hons), *Stellenbosch*, Postgraduate Student

Catherine Sutherland, BSc(Hons) *UAdel.*, Postgraduate Student (concluded duties 9/5/00)

Christopher Smyl, BSc(Hons) *UAdel.*, Postgraduate Student

Nicholas Andrew Yap, BSc(Hons) *UAdel.*, Postgraduate Student

Kate Susan Howell, BSc(Hons) *UNSW*, Postgraduate Student (commenced 22/5/00)

Nadia D'Incecco, BAgSc(Food Tech)(Hons) *Padova*, Visiting Italian Postgraduate Student (commenced 6/12/99)

Kerry Leigh Wilkinson, BSc(Hons) *Flinders*, Postgraduate Student (commenced 21/3/00)

Carolyn Jane Puglisi, BSc *Flinders*, BSc(Hons) *UAdel.*, Postgraduate Student (commenced 28/3/00).

Duane Coates, BSc *UAdel.*, Postgraduate Student

Helen McCarthy, BAgSc *UAdel.*, Honours Student

Nina Viergutz, BAgSc *UAdel.*, Honours Student

Marie Pearce, BAgSc *UAdel.*, Honours Student

Industry Services team members*

Peter William Godden, BAppSc (Wine Science) *UAdel.*, Winemaker/Manager Industry Services

Mark Gishen, BE(Chem) (Hons) MEngSc(Chem) *UMelb.*, Quality Liaison Manager

Alexander Nikolai Sas, BSc(Agric) (Hons) *UWA*, Viticulturist (concluded duties 26/11/99)

Sally-Jean Bell, BSc(Hons) *UWA*, GradDip(Wine) Roseworthy, PhD *UWA*, Viticulturist (commenced 28/2/00)

Adrian Dermott Coulter, BSc *Flinders*, GradDipOenol, *UAdel.*, Oenologist

Peter Valente, BSc(Hons) *Flinders*, Chemist

Ella Margaret Clare Robinson, BA, BSc(Hons) *UAdel.*, Chemist (commenced 2/8/99)

Raelene Joan Blair, CertAppMgt(Marketing) *AIM*,

Communication and Publicity Manager/Personal Assistant to the Director

Creina Standish Stockley, BSc(Hons) *UAdel.*, MSc *Flinders*, MBA *USA*, Health and Regulatory Information Manager

Catherine Grace Daniel, BA *ANU*, GradDip(Lib) *RMIT*, Librarian

Ingrid Betty-Maud Oats, DipLibInfo *Adel. Tafe*, Library Technician

*Several members of the Industry Services Team lead or take part in a number of research projects.

Analytical Service

Donald Robert Buick, BSc *UAdel*, Grad Dip(BusAdmin) *USA*, AIFST, Manager – Analytical Service (commenced 14/2/00)

Susan Mary Weeks, GradCertMgmt *USA*, Manager—Analytical Service (concluded duties 8/11/99)

John Benjamin Hughes, DipWineMrktg *UAdel.*, Analytical Service Supervisor – Administration

Matthew Grant Holdstock, BSc *Flinders*, Analytical Service Supervisor – Laboratory

Gregory Andrew Ruediger, BAppSc *SAIT*, GradDipOenol, *UAdel.*, Trace Analysis Laboratory Supervisor

Kevin Herbert Pardon, AssDip(AppChem) *SAIT*, Analyst

Andrea Dale Kemp, AssDip(Farm Mgmt), *Roseworthy*, BA, *UAdel*, Casual Analyst

Randell Leith Taylor, BSc(Hons) *UAdel*, Casual Analyst

David Rolfe Boehm, BSc *UAdel*, Casual Analyst

Gayle Ann Baldock, BSc(Hons) *Guelph*, Technical Officer/Casual Analyst

Amanda Louise Cook, AdvCert (Lab Tech) *Mackay*, Casual Laboratory Technician (Maternity leave from 17/2/00)

Radka Kolouch, AssDip(Food Tech) *Czech Republic*, Casual Laboratory Technician

Matthew James Cream, Casual Laboratory Technician

Jeremy Crispin Hack, Casual Laboratory Technician

Adam Richard Loveys, Casual Laboratory Technician

Bao Tran, AssocDipChemTech *USA*, Casual Laboratory Technician

Sandra Lloyd-Davies, BA *Flinders*, Casual Laboratory Administration Assistant

(commenced 24/1/00)

Administration

Janet Currie Currie, BA *Glasgow*, MAICD, Company Secretary

Sita Soetratma, BBus(Mgmt) *USA*, Accountant

Carolyn Debra Grant, AssDip(Acc) *Panorama Tafe*, Secretary to the Company Secretary/Conference Secretariat (commenced 6/12/99)

Narelle Elizabeth D'Costa, Accounts Clerk

Heather Margaret Donnell, Secretary to the Director (commenced 1/11/99)

Emma-Kate White, Receptionist

Maria Concettina Mills, Casual Receptionist

Jodi Anne Slade, Cleaner



Highlights of the year

It was demonstrated that additional oak flavour can be generated from precursor forms during bottle ageing.

The use of mass spectrometry to differentiate major grape varieties using their complement of unstable proteins is feasible. The mass-profiles of proteins in different juices from distinct varieties showed significant differences and these differences were consistent from year to year and across regions.

The ability of NIR to rapidly measure berry colour and other quality parameters has been firmly established, with excellent cooperation with numerous industry partners making this a truly collaborative project. The outlook for development of affordable instrumentation for widespread use is promising.

There is a rapid and early loss of volatile flavour compounds when wine is stored in bag-in-box packaging. The extent of the loss varies widely from compound to compound.

The searchable database of the John Fornachon Memorial Library was made accessible to Australian winemakers and grapegrowers from remote locations via the internet.

Our Analytical Service showed strong revenue growth, particularly in contract sensory evaluations, and saw the introduction of several new services including that for ochratoxin A.

Combining heat treatment with the addition of proteolytic enzymes reduced the concentration of unstable protein in white wine and the bentonite addition required to achieve stability was halved. There appeared not to be any significant sensorial impact of the heat treatment.

A spectrophotometer has been modified to measure browning, the result of oxygen permeation, whilst the wine is in the bottle. This non-destructive method allows individual bottles to be tracked throughout an experiment and through their storage.

The aroma impact of ascorbic acid addition to white wines was shown to be minor. After six months storage there was no detectable difference in aroma between white wines bottled with and without ascorbic acid addition. The influence on pinking susceptibility is still to be addressed.

Rapid methods to quantify the levels of tannins in seed extracts and in wine have been developed. These methods are a significant advance on what is currently available because the tannins are clearly separated from other compounds in wine and their identity has been rigorously demonstrated rather than inferred.

To assist our flavour research work, stable isotope dilution methods have been fully validated for 13 wine components derived from fermentation or from the grape.

The Institute's improved sensory evaluation facilities have been extensively used in numerous tastings for Analytical Service, Industry Services and research experiments, with the level of expertise of Institute panels continuing to develop. A set of formal sensory procedures were established to test grape berry homogenates for specified characters. The formal study clearly establishes that black pepper flavour can be tasted and scored in berries. The methods developed for tasting of berry samples by a trained panel could be extended for other purposes, for example for quality assessment of grapes.

Requests for information from the John Fornachon Memorial Library staff increased by 206% over the previous year.

Institute staff co-authored 33 publications; gave 59 seminars/talks (excluding the Roadshow seminars); presented 11 posters, conducted 3 workshops; gave 30 lectures and supervised 26 students during the year.

Six senior staff spent a period of six days on a Roadshow to Canberra and NSW where more than 40 seminars on a variety of topics were presented

The Advanced Wine Assessment Course was held in July and December, enhancing the analytical sensory skills of another 60 participants.

An Email Bulletin Service was made available to the Australian wine industry, providing a fast and cost efficient way of keeping industry aware of current technical issues.

The perception of diacetyl as a 'buttery' component very much depends on the individual wine in which it is presented.

Analyses of wines retained for approximately three years show that diacetyl is relatively stable in the bottle.

Institute staff hosted 197 international visitors during the year from countries such as Canada, China, Denmark, France, Germany, Italy, Japan, New Zealand, Portugal, South Africa, Spain, United Kingdom, United States of America and Zimbabwe.

The extension to the buildings of The Australian Wine Research Institute was officially opened by Dr Barbara Hardy AO on Tuesday, 26 October 1999 in front of 110 assembled guests representing many facets and regions of the Australian wine industry.



Staff activities

In addition to undertaking research projects described in this report, the Institute performs a large number of external activities in support of the Australian wine industry.

Information on seminars, talks and poster papers given to outside organizations, academic lectures delivered, graduate students supervised, and the papers published is tabulated and can be found in Appendixes 1–4 of the Annual Report. Activities in addition to those in the Appendixes are described below.

Peter Høj is a member of the following:

- Prime Minister's Science, Engineering and Innovation Council
- South Australian Innovation, Science and Technology Council
- International Trade and Technical Advisory Committee (AWBC)
- Compliance and Technical Advisory Committee (AWBC)
- Technical Committee (Winemakers' Federation of Australia)
- Wine Committee (Royal Agricultural and Horticultural Society of South Australia)
- Waite Campus Management Committee
- Committee of Management, Viticultural Publishing, publisher of *Australian Journal of Grape and Wine Research*
- Editorial board of the *Journal International des Sciences de la Vigne et du Vin*
- Conference Planning Committee of the Eleventh Australian Wine Industry Technical Conference (7-11 October 2001, Adelaide) (Chair)
- Australian Organising Committee of the 26th World Congress and 81st General Assembly of the Office International de la Vigne et du Vin (OIV) (11-18 October 2001, Adelaide)

He is also the Institute's representative on the following Adelaide University's Boards/Committees:

- Faculty Board
- Advisory Committee
- Management Committee of the Department of Horticulture, Viticulture and Oenology

Janet Currie is the Public Officer of the Australian Wine Industry Technical Conference Inc.

Rae Blair is a member of the Conference Planning Committee and is the Treasurer and Conference Manager of the Eleventh Australian Wine Industry Technical Conference. She is also a member of the Australian Organising Committee of the 26th World Congress and 81st General Assembly of the OIV.

Creina Stockley is a member of the Australian Wine and Brandy Corporation's International Trade and Technical Advisory Committee and the Legislative Review Committee, a member of the Technical Committee of the Winemakers' Federation of Australia and the Australian delegate for the OIV Nutrition and Health Sub-commission.

Elizabeth Waters is Leader of Program 1 of the Cooperative Research Centre for Viticulture II.

Mark Sefton is on the editorial review board of the *International Journal of Vine and Wine Sciences* and is the project leader of project 1.3 of the Cooperative Research Centre for Viticulture II.

Leigh Francis is an Editorial Board member of the *Journal of the Science of Food and Agriculture*, Editorial Review Board member of the *American Journal of Enology and Viticulture*, Chairman of the Royal Australian Chemical Institute (SA Branch), Medicinal and Agricultural Chemistry Group. He is Leader of Project 1.4 of the Cooperative Research Centre for Viticulture II.

Ken Pocock serves on the Waite Campus Occupational Health and Safety Coordinating Committee.

Paul Henschke was member of the IUMS Congress of Mycology Symposium Planning Committee (August 1999), member of the Yeast Products and Discovery organising committee and is the Convenor of Microbiology Subgroup of The Interwinery Analysis Group. Paul Henschke served as Program Leader of Program 6 in the Cooperative Research Centre for Viticulture I. He serves on the Editorial Review Board of the following journals: *Australian Journal of Grape and Wine Research*; *American Journal of Enology and Viticulture*; and *Australia and New Zealand Wine Industry Journal*. Paul Henschke acted as dissertations examiner for the University of New South Wales.

Peter Godden is a member of the Conference Planning Committee and is the Workshop Convenor of the Eleventh Australian Wine Industry Technical Conference and was on the organising committee of the International Cool Climate Symposium (Melbourne, January 1999).

Mark Gishen assisted with the program development for the Sixth Australian HACCP Conference (Adelaide, August 1999).

Matthew Holdstock is a member of the Interwinery Analysis Group Inc.

Visitors to the Institute

Australia

- Simon Banks, Senior Adviser to the Shadow Attorney-General, David Cox, Federal Member for Kingston, Alison Turner, Chief Executive Officer, National Registration Authority, Vic Edge, Deputy Chief, NSW Agriculture (17 June 1999)
- Brian Mills, International Agreements, Portfolio Policy and International Division, Department Agriculture, Fisheries and Forestry, Australia (22 July 1999)
- André Boucard, Chief Executive Officer; Fabrice Audan, Finance and Administration Director; Stephen Couche, Sales and Marketing Director; Philip Laffer, Viticulture and Winemaking Director; Carey Weston, Export Director; Roger Meeks, Technical Director; Brian McGrath, Sales Director; Bernard McCallum, Human Resources Director; Dr Don Lestor, National Viticulture Manager; Annie Sloan, P.A. to

Viticulture and Winemaking Director, Orlando Wyndham Group (29 June 1999).

- Rhett Marlowe, Guy Darling, Bruno Brombal, Mike Stone, Grieg Greenwood, Brian Englefield, Leo Pech, John Dal Broi (25 August 1999)
- Professor T. Norman Palmer, Pro Vice-Chancellor Research and International, James Cook University of North Queensland (13 October, 1999)
- Dr Neil Barnett, Associate Professor Analytical Chemistry, School of Biological and Chemical Sciences, Deakin University, Victoria (8 November 1999)
- Tony Gilson, Noel Heyes, Cork & Seals (24 November 1999)
- Murray Arthur-Worsop and Ben Sammy, South Australian Department of Industry and Trade, (22 December 1999)
- Lewis Tessarolo, DSM (Gist-brocades) (24 January 2000)
- Graeme Anderson, Lallemand (24 January 2000)
- Kym Ludvigsen, Southcorp Wines (25 January 2000)
- Mark Forbes, Australian Water Quality Centre (15 February 2000)
- Professor Noel Dunn, Director, and Dr Rachel Lucas, Business Manager, Cooperative Research Centre for Food Industry Innovation (15 February 2000)
- Mr Andrew Kleinig, Southcorp Wines (16 February 2000)
- Mr Paul Croxton of Boulevard Nurseries Mildura (28 February 2000)
- TAFE students (13 March 2000)
- Mr Kevin Pfeiffer, Australian Vintage (22 March, 2000)
- Members of the Prime Minister's Science, Engineering and Innovation Council: Dr Colin Adam, Professor Brian Anderson, Ms Stella Axarlis, Dr Robin Batterham, Mr David Buckingham, Professor Ian Chubb, Mr Martin Cole, Dr John Keniry, Senator the Hon Nick Minchin, Professor Don Nicklin, Ms Johanna Plante, Professor Vicki Sara, Professor Sue Serjeantson and guests of the Committee: Professor Helen Garnett and Mr Rod Bishop (19 April 2000)
- Ms Tammy Pavelic, Ms Katherine Schmutter, Mr George Shaw, Mr Ian Shortt, Department of Industry, Science and Resources (19 April, 2000)
- Mr Richard Heyneman, South Australian Department of Industry and Trade (20 April and 16 May, 2000)
- Simon Jongebloed, Invest South Australia (1 and 16 May 2000)
- Andre Lombard and Chris Malan, Netafim (17 May 2000)
- Dr Lucy Baker, Materials Performance Technologies (18 May 2000)

Canada

- Associate Professor Ken Froese, University of Alberta (15 February 2000)

China

- Yeujin WANG, Northwest Agricultural University; Wang YONGLIN, China-

Czech Friendship Farm winery; Cui YANZHI, China-Czech Friendship Farm winery; Li ZELIN, Hebei Provincial State Farm Bureau; Liu YANLIN, Interpreter and lecturer (22 July 1999)

Denmark

- Professor Klaus Grunert and Associate Professor Hanne Harmsen, Centre for Market Surveillance, Research and Strategy for the Food Sector, The Aarhus School of Business (6 April 2000)

France

- Professor Roger Bessis, Dijon (25 June 1999)
- Claude Espeillac, Lallemand, Toulouse (19 July 1999)
- Patrick Ricard, Chief Executive Officer and Chairman; Laurent Lacassagne, Finance Director; Jean-Manual Spriet, Plan/Budget Director; Tim Paech, International Marketing Manager, Groupe Pernod Ricard, Paris, France (29 June 1999)
- Professors Michel Feuillat and Claudine Charpentier, Jules Guyot Institute of Grapes and Wine, University of Bourgogne, Dijon (25 August 1999)
- J.P. Gaudillere, INRA, Bordeaux (27 August 1999)
- Laurent Dulau, Lallemand (24 January 2000)
- Patrice Pellerin, Application Research Manager Wine Ingredients, DSM-Food Specialties Oenology, Gist-brocades, Servian (24 January 2000)
- Bruno Freisen, Martin Janz, Jean-Baptiste Traversac, delegates from the International Cool Climate Symposium (25 January 2000)
- Denis Fetzmann, Domaine Louis Latour (14 February 2000)
- Dr Marc Dubernet, Dr Marie Odile, Sylvain Coulomb, Laboratory Dubernet (16 February 2000)
- Mr Robert Boidron, Director and Mr Gilbert Peyrant of ENTAD in Montpellier (28 February 2000)
- Dr Eric Degryse, Chef du Laboratoire de Microbiologie, Pernod Ricard (23 May 2000)

Germany

- Dr Sibylle Krieger, Lallemand Danstar Ferment AG (12 May 1999)
- Professor Manfred Grossmann, Head of the Department of Microbiology and Biochemistry, The Geisenheim Research Institute, Geisenheim (23 August 1999)
- Dr Wolf Sponholtz, Forschungsanstalt für Weinbau Gartenbau Getranketechnologie und Landespflege, Geisenheim (24 January 2000)
- 35 Viticulture students from Geisenheim (31 January 2000)
- Dr Albert and Maria Paul, Ralf Hirschfield, Otto Jopp, Dieter Kramer, Asmus Schmidt, Heinz Trogus, Otto and Otti Schaal, Ursula Koch, Sonja Andreus (22 February 2000)
- Dr Jens Jäger, Director R&D, Ohlinger Quality Corks and Filtration (5 April 2000)

Italy

- Dr Giovanni Martelli, Università degli Studi di Bari (9 March 2000)

Japan

- Eiichiro Nakajo, N.I. Corporation, Masahide Sakakibara, Sapporo Wines Ltd (22 March, 2000)

New Zealand

- Associate Professor David Heatherbell, Lincoln University, Christchurch (25 January 2000)
- Dr Valerie Linton, Materials Performance Technologies, Lower Hutt (18 May 2000)

Portugal

- Jochen Michalski, Cork Supply Group (26 May 1999)
- Antonio de Barros Amorim, L. Ferreira, Amorim Group (24 November 1999)
- Mr Manual Santiago, Export Manager, Juvenal S.A. (5 April 2000)

South Africa

- Chris Kelly, Winemaker, Stellenbosch Farmers (2 December 1999)
- Ms Roleen Carstens, Ms Marika van der Merwe, ARC-Fruit, Vine and Wine Research Institute, Stellenbosch (14 March 2000)
- Mr Sol Bezuidenhout, Researcher – Centre for Process Engineering, University of Stellenbosch (4 April 2000)
- Danie Malan, Cristof Reinhold, Etienne Buys, Frikkie Koegelenberg, Chris Venter, Brian Stipp, Andrew Lambrechts, Neil Rossouw, Hannes Erasmus (17 May 2000)

Spain

- Elena ADELL SAN PEDRO; Montserrat ALMONACID MARTINEZ; Ana ARAZURI IRIGARAY; Natividad ARENILLAS GARCIA; Luis ARROYO FELICES; Lorenzo AYUSO LLORENTE; Ma Francisca BALLESTEROS SALINAS; Jose Maria BARRERO GONZALEZ; Jose Daniel BELDA ALBERO; Victoria BENAVIDES AGUNDEZ; Ma Luisa BENGOCHEA GARCIAINDIA; Evaristo CARDELL CHRITELLYS; Juan CARRION TOMAS; Ma Dolores CASADO SANZ; Jose Alberto CASA ASIN; Amelia COLOMA GANAN; Edmundo DE DOMINGO GONZALEZ; Juan Jose DIAZ OCANA; Almudena DIEZ DE LA MORENA; Miguel DIEZ DE LA MORENA; Dionisio DIEZ HORMILLA; Angel ESCOBAR DE LA TORRE; Franciso Javier ASCOBAR DE LA TORRE; Sebastian FLORES FLORES; Enrique GARCIA MARTINEZ; Jose Carlos GARCIA VEGA; Jose Manuel GARCIA DE LA CUADRA ARIZO; Pablo GONZALEZ OSSORIO; Alfredo GONZALEZ VIVANCO; Cesar GUTIERREZ HINOJAL; Valerio Luis GUTIERREZ AFONSO; Jose Alberto GUTIERREZ HINOJAL; Juan HUERTA MARTINEZ; Ignacio CAMIO AIZPURUA; Josefa LOPEZ GONZALEZ; Alfredo MADRIGAL LLORENTE; Salvador MANJON ESTEA; Roberto MARTINEZ SANZ; Montserrat MOLINA MASO; Margarita MORENAS VICENTE; Antonio Miguel NAVARRO MUNOZ; Jose Luis NUNEZ DENIA; Javier OCON GALLEGGO; Caso PEQUENO BORREGO; Jose Manuel PNEDO CONTRERAS;

Antonio Jose PINERO GIL; Fernando POZO REMIREZ; Ana Isabel QUINTELA SUAREZ; Vicente SANCHEZ-MIGALLON SANCHEZ-GIL; Marco Antonio SANZ MARTIN; Antonio SANZ MORO; Pedro Antonio SARRION MARTINEZ; Manuel SEVA LAZARO; Ma Jesus SOLER SANCHIS; Concepcion SOUCASE GONZALEZ; Jose Mario VEGAS ARRANZ; Ma Pilar VEGAS CORDOBES; Rafael VICENTE NAVARRO; Pilar VINUESA LECUONA; oenologists (4 June 1999)

- Pedro Contreras, winemaker Vina La Fortuna; Lontue and Marcelo Retamal, winemaker Vina Santa Ines, Isla de Maipo (30 November 1999)

United Kingdom

- Gideon Rachman, Britain Editor, *The Economist*, London (27 October 1999)
- Rosemary Harris, Director, Private Liquor Brands Ltd, West Sussex (15 November, 1999)
- Professor Colin Dennis, Campden & Chorleywood Food Research Association Group, Gloucestershire (1 May 2000)
- Dr Richard Pugh, Leatherhead (16 May 2000)

United States of America

- Dr Terry Lee, Vice President Research, E&J Gallo Winery, Modesto (14 July 1999)
- Donald Frederick, Journalist, Washington, D.C. (25 October 1999)
- Dr Ronald Wrolstad, Professor, Department of Food Science and Technology, Oregon State University (17 November 1999)
- Jane Robichaud, Director Winemaking Research/Sensory, Beringer Wine Estates, St Helena, California (14 January 2000)
- Dr William Adams, Rick Dunst, Lowell and Pauline Ford, Charles and Hannah MacGregor, Dr Dave Miller, Frank Mitchell, Susan Meredith, Wolfgang Moritz, Myron Redford, Vikki Wetle, Dr Russell Smithyman, Dr Sara Spayd, Ray and Patricia Straughan, Dr Julie Tarara, Phillip Throop, Professor Robert and Mrs Wample, Ivan and Martha Wells, delegates from the International Cool Climate Symposium (25 January 2000)
- Dr Sara Spayd, Food Science, Enology, Washington State University (1 February 2000)
- Professor Ralph E. Kunkee, University of California, Davis, William J. Nakata, ASV Wines Inc., Delano (25 February 2000)
- Dr Barbara J. Mazur and Dr Scott Tingey, DuPont Agricultural Enterprise, Delaware (5 April 2000)
- Dr Terry Lee, Vice President Research, E&J Gallo Winery, Modesto (5 June 2000)

Zimbabwe

- Samuel Pfidzayi, Director Wineries, Cairns Wineries, Marondera, Zimbabwe and Phil Laing, Finance Director, Cairns Holdings Limited, Harare (26 November 1999)



Scientific Research Teams' Reports

Analysis of quality parameters in grapes and wine using near infrared spectroscopy

Staff: Dr Leigh Francis, Mark Gishen, Dr Bob Dambergs (BRL Hardy), Dr Michael Esler, Dr Bruce Kambouris (BRL Hardy), Dr Wies Cynkar, Professor Peter Høj, Nathan Schumacher (BRL Hardy), Dr Sally-Jean Bell, Peter Godden, Patrick Iland (Adelaide University), Russell Johnstone (Orlando Wyndham), Dr Andrew Kleinig (Southcorp Wines), Dr Eric Wilkes (Rosemount Estates), Ben Zander (Orlando Wyndham), Inca Lee (Orlando Wyndham), Nick Yap

Following the outcomes of our initial pilot project on Near Infrared (NIR) spectroscopy in 1998/1999 and its promising leads towards providing very fast, low cost analyses of a range of parameters important to commercial wine production (see Institute Annual Report 1999), an increase in funding was received from both the GWRDC (AWR 98/2) and the CRCV (CRCV 1.4). This allowed us to employ a dedicated post-doctoral researcher (Dr Michael Esler) with previous experience in development of spectroscopic calibrations and to support this role efficiently with the transfer of Dr Wies Cynkar to support Dr Esler. Dr Esler performed his PhD working on Fourier Transform Infrared Spectroscopy (FT-IR) for the rapid determination of gases in atmospheric air.

The project has progressed quickly, with the close involvement of several industry partners helping to accelerate the research, notably teams led by Dr Bob Dambergs (BRL Hardy), Mr Russell Johnstone (Orlando Wyndham), Dr Andrew Kleinig (Southcorp Wines) and Dr Eric Wilkes (Rosemount Estates). This involvement of wine companies has been particularly pleasing and fruitful as it not only has involved in-kind contributions but also cash commitments invested in a spirit of cooperation.

A real collaboration between wine companies, a specialist instrument maker and the Institute has been established with the prospects of tangible practical outcomes being delivered at an accelerated rate for the benefit of the wider industry.

NIR 6500 analyser

Links have been established with the Grains Industries Centre for NIR, as it is recognised that the grains industries have been involved with NIR technology for many years and the wine industry can learn from their experience.

This project has had the overall aims of:

- development of an NIR method for determining colour, total soluble solids and pH in grapes suitable for practical industry use;
- investigate the ability of NIR to measure other parameters in grapes and wine: the glycosylated flavour precursors (G-G), yeast assimilable nitrogen, phenolic compounds and the degree of botrytis infection; and
- develop an NIR method for determining methanol in spirits.

Berry colour, pH and total soluble solids

The consolidation of calibrations developed for analysis of grape berry colour, total soluble solids and pH by NIR scanning has continued, involving study of as wide a range of samples as possible. The berry samples are scanned with no preparation except for homogenisation using a high speed laboratory homogeniser. Many hundreds of 1999 and 2000 season homogenate samples (predominantly Shiraz, Cabernet Sauvignon and Chardonnay from the Riverland, Barossa Valley and South East SA regions) have been analysed by the conventional laboratory method and by scanning using research grade NIR instruments located at BRL Hardy (Berri) and the Institute. The cooperation of industry partners (BRL Hardy, Orlando Wyndham, Southcorp, Rosemount Estates) has meant that more than 1500 berry samples from a wider range of South Australian, New South Wales and Victorian viticultural regions were also obtained from the 2000 season. The AWRI instrument will be used as a 'master' instrument, and in the near future the likelihood of transference of calibrations across instruments will be tested. The ability to readily transfer data from instrument to instrument is a necessary prerequisite for practical application of NIR spectroscopy across the industry.

As expected from earlier data, good NIR calibrations have been developed. Evaluation of the robustness and accuracy of the calibrations has been an emphasis this year, to ensure that any calibration



Left to right: Dr Leigh Francis, Mark Gishen and Dr Michael Esler

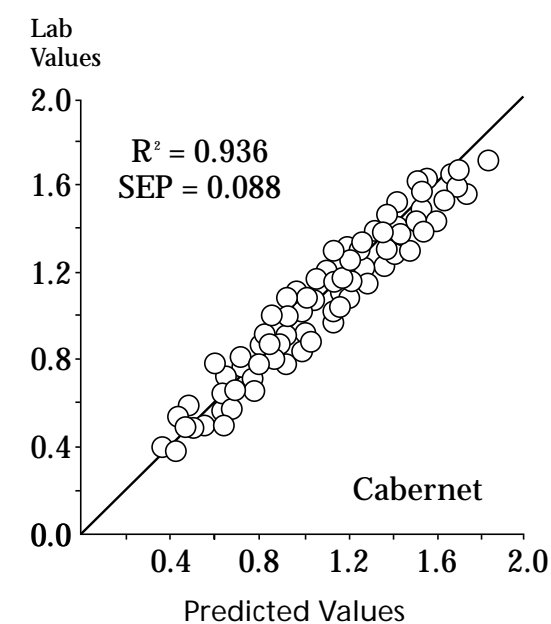


Figure 1.
Colour of red grape homogenates (mg/g) NIR Systems 6500

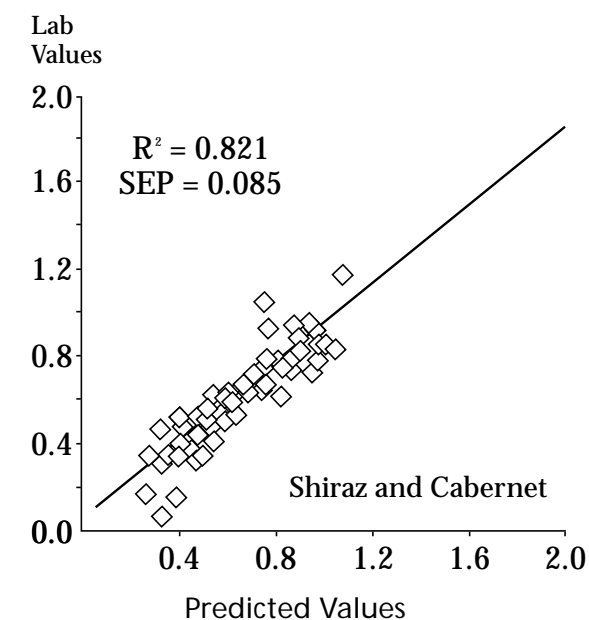


Figure 2.
Colour of red grape homogenates (mg/g) Prototype



Scientific Research Teams' Reports

developed can be confidently applied to appropriate samples. It has been found that while the calibrations hold across seasons, best accuracy is achieved with a calibration produced from one season's samples alone, and a similar situation may exist with variety and region variables: a calibration can be developed that will give acceptable results for samples from multiple regions or from several varieties, but best accuracy seems to be obtained from single variable data sets. From a data set of approximately 5,000 Cabernet Sauvignon, Merlot and Shiraz samples, it was found that the best colour calibrations were obtained from separated varieties. The significance of this finding depends on the accuracy to which determinations are required to be made.

A set of Shiraz samples from the 1999 season was the most difficult calibration set: samples with a colour value greater than 1.1 mg/g needed a separate calibration from the less coloured samples to be predicted accurately. This observation may be related to the physiology of Shiraz berries with often acute shrivelling and associated degeneration of colour. Work was repeated with equivalent 2000 vintage samples, initially with vineyard samples, but then with load samples collected at the weighbridge (collected as hand sorted whole berries). This 2000 season Shiraz sample set did not show signs of requiring a separate high/low calibration except for samples at the very top of the colour scale (1.8 mg/g). Grapes with such high colour content represent a very small proportion of samples.

The question of matrix effects influencing the calibration needs to be examined further, and may cause inconvenience regarding the routine application of NIR in winery labs. It is possible that a decision may need to be made weighing up the advantages between achieving a certain level of accuracy from a 'universal,' already developed calibration compared to the amount of effort required to improve the accuracy further. In the worst case, it may eventuate that a sample set must be analysed with the lab method as well as scanned by the NIR instrument for samples from each viticultural region, each variety and, most importantly, each year in order to develop a sufficiently accurate calibration. From current knowledge this is not likely, as reasonable measurement accuracy can be obtained from an existing calibration, and

with further investigation it is expected that this issue will be overcome.

If calibration transfer is achievable between instruments, the large sample sets scanned in this project will be invaluable. A likely scenario for provision of NIR calibrations will be that a database of spectral information will be held at the Institute for industry use, to ensure accurate calibrations will be available for samples of disparate origin.

Accurate berry colour, soluble solids and pH calibrations can be obtained for berry samples, from a range of varieties and regions.

A potential requirement for separated calibration sets for improved accuracy remains to be confirmed.

With large numbers of samples to analyse, the homogenisation step used in the current method becomes an issue, with this step taking significant time to carry out (of the order of five minutes per sample). Preliminary work with an instrument that can scan whole fruit looks promising but seems to work well for colour only, not soluble solids and pH.

Scanning whole berries may be feasible for colour measurement.

Five identical, prototype NIR instruments which can be acquired at relatively low cost have been trialled by the project collaborating partners, and results to date indicate that these instruments can analyse grape homogenates to an acceptable level of accuracy. The likelihood of transferring calibrations between these instruments is being addressed currently.

It is not necessary to use research quality NIR instruments to achieve acceptable measurements for berry colour, soluble solids and pH measurement.

Investigations have been carried out with Orlando Wyndham to assess the suitability of these lower cost instruments with a fibre optic probe attached to measure bins of machine harvested fruit. The measurement of machine harvested fruit directly in bins introduces additional challenges, the load being non-homogenous, and containing matter other than grapes. However, by immersing the probe directly in a bin sample, laboratory scale experiments have shown that the instruments may be suitable for this application, although calibrations at this early stage of developments were

understandably not of the same degree of accuracy as obtained from scanning homogenised samples. This work indicated the number of scans that will be required to measure a whole bin, and issues were identified as to the practicalities of developing calibrations with bin samples.

NIR has potential to directly assess grape loads in bins at the winery weighbridge, but further work is required to increase the accuracy and practicality of the technique.

Other analyses

The use of NIRS for the rapid determination of glycosyl-glucose (G-G) in berry homogenates has been pursued, with a data set of 100 Cabernet Sauvignon berry homogenates providing good calibration results for this parameter. However, a relatively poor calibration was obtained from a set of 154 Chardonnay samples. This result may have been due to a delay between scanning the samples by NIR and then conducting the laboratory analyses, so it was decided to analyse a further set of 40 Chardonnay samples from the 2000 season, with the scanning and analyses carried out at the same time. For this most recent set the results were satisfactory, with an accuracy comparable to the lab method. Further samples will be analysed to establish definitively the practicality of analysing this parameter in white grapes by NIRS.

For other applications such as nitrogen status in juice and phenolics in grapes and wine, small sample sets have been analysed with some encouraging results.

The ability of NIRS to measure methanol and also ethanol in spirits has been definitely established and a manuscript is in a late stage of preparation.

Viticultural control of flavour compounds in wine

Staff: Dr Mark Sefton, Dr Leigh Francis, Dr Gordon Elsey, Stella Kassara, Dr George Skouroumounis, Professor Peter Høj, Professor Rolf Prager (Flinders University), Carolyn Puglisi, Gayle Baldock, Yoji Hayasaka, Dr Josephine Newton, Dr Wies Cynkar

This project has, with the emergence of the CRCV, changed its name from 'Grape composition and wine flavour' to 'Viticultural control of flavour compounds in wine' to reflect the increasing vision of the need to develop tools that will allow us

to set and achieve sophisticated quality specifications of grapes. The project, with a dual funding base (AWR 6 and CRCV 1.3), has the overall aims of achieving:

- the identification of grape flavour components which impact on wine quality;
- development of routine analytical methods for measuring these components accurately; and
- knowledge of the impact of viticultural practices on the levels of grape flavour constituents.

The longer term objective of this project, stemming from the achievement of the above aims, is to allow producers to better control levels of desirable and undesirable wine flavours arising from the grape berry, and to be able to set specifications for particular wine styles.

Black pepper aroma in Shiraz wines

This aspect of the project has the objective of providing winemakers and viticulturists with the ability to manage the level of black pepper flavour in Australian Shiraz wines. It also serves as a pilot program for our wider goal of identifying and measuring key wine compounds with the aim of being able to specify viticultural and oenological parameters of importance to the characteristics of the bottled wine. Work carried out to date aims at identifying the compounds that cause this distinctive aroma character. Shiraz grape berry samples with black pepper flavour have been studied, together with grapes that do not show this flavour.

Gas chromatography-mass spectrometry (GC-MS) studies have been carried out in parallel with sensory investigations. Shiraz de-seeded berry homogenates from two Coonawarra vineyards were tasted under controlled conditions by a trained sensory panel and rated as high in pepper flavour, with two other samples from different vineyards rated as low in pepper.

A set of formal sensory procedures were established to test grape berry homogenates for specified characters. The formal study clearly establishes that black pepper flavour can be tasted and scored in berries. The methods developed for tasting of berry samples by a trained panel could be extended for other purposes, for example for quality assessment of grapes.



Initial GC/MS investigations showed that a dynamic headspace trapping procedure was most suitable for analysis of these samples. From further GC/MS and GC/Olfactometry (GC- 'sniff') studies, it appears that several volatile compounds may be involved in the pepper flavour attribute in berries, and a list of sesquiterpene and monoterpene compounds present at higher concentration in the high pepper samples has been developed. Several of these compounds are reported to be involved in the aroma of black peppercorns. Current investigations with 2000 season samples are being undertaken to find how the compounds tentatively implicated in the pepper flavour in Shiraz berries and wines vary in concentration across a range of samples, and confirming whether the levels observed relate to the intensity of pepper flavour.

Development of an accurate, rapid analytical method for the quantification of key wine flavour compounds

As previously reported, a stable isotope dilution analytical method is under development for quantifying known key grape-derived wine flavour compounds. The establishment of this method will mean grape and wine samples will be able to be analysed routinely for a set of specific flavour compounds. Most of the flavour compounds must be quantified at microgram per litre levels or below so the analysis must be sensitive, accurate and precise. The method, once completed, will enable future research on viticultural and oenological controls of flavour formation, and will be added to over time with further compounds identified from the literature or from on-going research. Targeted compounds included to date are: β -damascenone (responsible for honey, cooked apple flavours), alpha- and beta-ionone (raspberry, violets), isobutyl methoxypyrazine (vegetative, capsicum-like) and the monoterpenes geraniol, linalool, and nerol (contributing to floral and citrus flavours). For each of these compounds, stable isotope labelled analogues have been prepared for use as internal standards for the method. Furthermore, fermentation ester flavour compounds have been added to the assay, so that an integrated approach of quantifying both grape derived and yeast

GC/MS with tray samples

derived compounds can be taken. The synthesised labelled flavour compounds have been incorporated into the analytical method, and have been used by the 'flavour scalping' project (discussed later in the report from the Industry Services team). Further optimisation and validation of the analytical method is still required.

Dr Gordon Elsey was recently appointed to a Postdoctoral Fellow position, and a student, Ms Carolyn Puglisi, has also recently commenced studies. Several grape components that have been observed in previous investigations are in the process of being synthesised by Gordon and Carolyn in order to be able to test what their impact may be on wine flavour. The availability of a much enhanced repertoire of analytical methods will also allow researchers to gain more information about the influences of viticultural treatments on berry flavour characteristics. Currently, much viticultural experimentation could yield substantially more information than is currently the case. With the availability of an extended bank of assays for grape and wine flavour compounds, more information about the outcomes of viticultural manipulations can be gained with relatively little effort.

A concerted effort to use organic chemistry for the investigation of suspected grape and wine flavour compounds has been strengthened. The subsequent development of assays for these compounds, together with the new knowledge of their sensory significance, will enhance the information that can be derived from much viticultural and oenological experimentation worldwide. To enhance our capacity in this regard, we now have CRCV-funded staff based in the Department of Chemistry at Flinders University in Professor Rolf Prager's group.

G-G Automation project

Following substantial delays in the delivery of an automated robotic system from an independent Adelaide firm, an independent review of the robotic system has been initiated. A Perth based engineering company was chosen to perform the review following a recommendation from a leading automation research engineer and as a result of an assessment of the background and capabilities of the company. A final report is not yet at hand.

Independent assessment of the system by Dr Francis has indicated that progress was made in the past financial year on this long-



Left to right: Dr Mark Sefton and Dr Gordon Elsey

running project initiated in 1995 as part of the previous Cooperative Research Centre for Viticulture.

Evaluation of an aroma sensing instrument to differentiate grape and wine samples

Assessment of the potential of a mass spectrometry based chemical sensor instrument, 'chemical sensor,' located at the Adelaide University's Department of Horticulture, Viticulture and Oenology has been continued for a number of applications. The instrument has the advantage of minimal sample preparation and rapid (approx. five minutes) results, compared to existing GC/MS methods. The system gives a fingerprint of the mass spectral fragmentation pattern from the total headspace of a sample, i.e. there is no individual quantification of particular compounds. The evaluation has been considered of lower priority than other research work, there being no current outputs or deadlines to be met. Thus the investigation has been carried out by Wies Cynkar during periods where there has been lesser demand from other projects, on average one or two days per month over the last year.

The potential use of the instrument for trichloroanisole taint has been the first application to be evaluated. From work with standard solutions of TCA added to model wine or white wine, the indications are that the instrument may have near sufficient sensitivity (to 5 ng/L) for detection of this compound. However, it remains to be confirmed whether with a cork wood background aroma or with a range of white

wine bases, the instrument will be a practical alternative to either a tasting panel or GC/MS analyses.

Wine sample sets have also been studied, and the indications are that the instrument can apparently differentiate among some sample types. For example, a blending experiment of commercially bottled Chardonnay and Riesling wines, indicated that the instrument could easily detect blends of 5%. Thus a 95% Chardonnay/5% Riesling blend was detectably different from a 100% Chardonnay. A range of commercial wines obtained from the last Adelaide wine show also showed differentiation, with some patterning among regions and varieties, but with a degree of overlap. This suggests that, as expected, volatile compounds present in commercial wines are not sufficient fingerprints to allow definite varietal or regional identification, but with further work it may eventuate that some variables can be consistently identified, such as, for example, year of production or broad variety identification. Careful thought needs to be made as to the applications that are appropriate with this technology, as well as the applications that may be of significant practical benefit to the industry.

Some preliminary work has been carried out with grape berry samples, primarily homogenates. While the signal from these samples was low, there were indications that a set of vineyard maturity samples of a range of ripeness levels gave different volatile profiles.

Currently, wines from the closure trial (further described on page 36) are being



Scientific Research Teams' Reports

analysed with the sensor, and the data related to the sensory panel results as well as other chemical composition data available.

The influence of oak cooperage on wine composition

Staff: Dr Mark Sefton, Alan Pollnitz, Dimitra Capone (extended leave from 25/2/00 to 31/12/00), Daniel Sejer Pedersen (until January 2000), Michael Raunkjaer (from February 2000), Dr George Skouroumounis

Additional oak flavour could develop during bottle ageing

A preliminary experiment on shavings of more than 20 oak samples indicated that after oak volatiles are totally extracted into model wine from oak shavings and the shavings are removed, these volatiles can continue to be generated, presumably from soluble precursor forms, also derived from the oak samples. A subsequent and more detailed trial based on a subset of 20 oak samples has been completed. It is clear that both oxidative and anaerobic processes contribute to this additional flavour generation.

The accumulation of oak components in wine during barrel ageing is not only a function of extraction rates but is also dependant on this hydrolytic generation. Additional oak flavour could also be generated during bottle ageing.

An experiment to determine whether coniferaldehyde can act as a precursor to vanillin in oak extracts, as has been suggested in the literature, has been completed.

The results clearly show that coniferaldehyde is not a significant precursor to vanillin, regardless of whether or not oxygen is present

These experiments constitute the final data to be incorporated into Mr Alan Pollnitz's PhD thesis. The thesis will cover virtually all of the stable isotope dilution analysis work on cork and oak components carried out over the past four years. Recent detailed accounts of the outcomes can be found in Institute publications #605, 623 and 627 and in a paper to be published in the *Australian Grapegrower and Winemaker* (July, 2000).

The origin of oak lactones, synthesis of potential precursors

It has been hypothesised that the gallic acid ester of the open chain form of the oak

lactones acts as a precursor to the oak lactones themselves. It is important to establish whether this could be so, as model hydrolytic studies then would yield valuable insights into whether the final oak lactone concentration of a wine could be modulated whilst keeping everything else equal.

Good progress has been made towards the synthesis of the gallic acid ester of the open chain form of the oak lactones. The protected form of the gallate ester has been synthesized, but the yield was very low. Model studies, which were undertaken to improve the yield of the esterification step, have shown that the yield of this step can be improved significantly, but because it proceeds slowly, competing relactonisation makes it necessary to first derivatise the acid function of the oak lactone in a reduced form. Such derivatisation has been successfully implemented, and the esterification of this modified form of oak lactone is now being undertaken.

Other matters

A preliminary experiment has been undertaken to investigate whether oxygen contributes to the effects of heating of fine shavings on oakwood composition. The results show that lignin breakdown by heat is more extensive when oxygen is present and that this results in greater amounts of vanillin, guaiacol and 4-methylguaiacol being formed in the oak compared to when oak shavings are heated in an inert atmosphere. The wines analysed for 4-ethylphenol and 4-ethylguaiacol were also analysed for other oak components.

There was no significant difference between varieties in the amount of vanillin or 4-methylguaiacol in 61 bottled single variety red wines. The concentration of vanillin in these wines ranged from 34 to 364 mg/L while that of 4-methylguaiacol varied between 1 and 26 mg/L. The concentration of guaiacol, which is formed during barrel toasting, varied from 10 to 72 mg/L. There was significantly more guaiacol in the Shiraz wines (mean concentration 38 mg/L) than the other varieties (mean concentrations of 20-23 mg/L). This is unlikely to be due to the use of more heavily toasted barrels in Shiraz winemaking, as the mean concentration of 4-methylguaiacol, which is also formed during barrel toasting, was virtually identical across the varieties. Guaiacol has been previously identified by the Institute

in hydrolysates of glycosidic fractions from Cabernet Sauvignon and Merlot grape samples, so it is possible that some of the guaiacol in the wines, particularly the Shiraz wines, was also derived from the grape.

On the basis of published sensory thresholds, the vanillin, guaiacol and 4-methylguaiacol is likely to have a limited impact only on the aroma and flavour of the 61 bottled wines for a majority of consumers.

In sensory terms the most important oak-derived compound is generally assumed to be the cis-oak lactone. Despite its apparent importance, however, the sensory threshold in wine of the naturally occurring form of cis-oak lactone (which is not commercially available) has never been determined. A threshold for a commercial mixture of racemic cis plus trans isomers has been reported to be 80 mg/L in a red wine.

All but one of the bottled wines contained oak lactones. As oak lactones have only been detected in wines that have been matured in oak barrels or treated with other oak products. It would appear that virtually all of these wines have also had some oak contact during their production. Both the absolute amount of cis-oak lactone and the cis/trans ratio varied widely in the bottled wines. The cis-isomer ranged in concentration up to 887 mg/L.

In red wines stored in a local winery in 44 American and 47 French new and used oak barrels from more than 20 suppliers, we observed cis/trans-oak lactone ratios of between 1 and 2 for the wines matured in the French oak while for those kept in American oak, the ratio varied from 8 to 10. These are in good agreement with the ratios reported by others.

Based on these observations, there appears to be some notable differences in oak usage between the bottled single variety wines analysed, with French (or other European) oak apparently favoured for the production of the Pinot Noir wines, while American oak seems to have been almost exclusively used in the maturation of the Shiraz wines. Some products are likely to have been aged in a mixture of both oak types. In several of the wines the ratio of cis- to trans-oak lactones was much higher than 10. This may simply indicate that the range of ratios in oak products is somewhat higher than indicated by previous trials. Alternatively, it

is possible that, during bottle maturation, cis-oak lactone is generated faster than the trans-isomer from precursor forms that have also been extracted from the oak. Such a mechanism for enhancing oak lactone concentration in wine is indicated by the results reported above.

Ms Kerry Wilkinson has accepted an offer of a PhD scholarship to work on the mechanism of formation of novel oak flavour compounds.

Studies on unstable wine proteins involved in haze formation

Staff: Dr Elizabeth Waters, Ken Pocock, Shauna Brown, Dr Miguel de Barros Lopes, Yoji Hayasaka, Professor Peter Høj, Dr Mark Sefton, Dr Leigh Francis

Previous work in this project focussed on characterising the proteins responsible for wine haze and the effect of irrigation and mechanical harvesting on the protein concentration of juice (Institute publications #384, 419, 488, 489, 492, 552, 562, 578, and 628).

Based on this knowledge, the project now enters its final stage of developing alternative methods to prevent haze formation and of exploiting the properties of the proteins to discriminate juices and wines variably.

Use of mass spectrometry to differentiate varieties

Since subtle differences between the molecular weights of proteins are readily detectable by electrospray mass spectrometry, it is theoretically possible to identify cultivars by the protein profile of berries, must and wine. Such an identification technique would complement and extend the identification service currently available, since DNA fingerprinting is valid for berries and must only. This has been explored.

Juices were collected from nine important white varieties and eleven important red varieties of grapes over at least two vintages and for eleven of these varieties from between two to four regions and their protein characteristics determined. The sensitivity and ease of operation of the technology was increased by using a so-called protein-trap method instead of the usual more lengthy reversed phase column chromatography methodology.



Left to right: Dr Zhong Kui Peng, Dr Sally Bell and Dr Elizabeth Waters

The data now including the 1997, 1998 and 1999 vintages showed clearly that the masses of grape juice proteins differed amongst varieties but was very consistent within varieties sampled across regions and seasons. The use of mass spectrometry of juice to differentiate the quantitatively most important grape varieties in Australia is feasible. It remains to be seen whether this technique can be extended to wine following conventional fining techniques. A paper describing these outcomes has been written and will be submitted for publication in September 2000.

Proteolytic enzymes and/or heat as alternatives to bentonite fining
Previous work at the Institute and elsewhere has shown that juice and wine proteins are resistant to proteolytic attack at temperatures below 25°C (Institute publications #364, 419, 489). At higher temperatures, other groups have reported losses of proteins and reduced bentonite requirements for juices and wines treated with proteases. Nevertheless, the use of proteases combined with heat treatment is not widespread in industry, possibly due to the perception that heating under any conditions is detrimental to wine quality. This appears to be unfounded, at least in some cases, because previous work at the Institute showed that short time (2 or 10 minutes) /high temperature (~90°C) treatment had no clear sensory effect on wine (Institute publication #444).

Preliminary trials with a range of commercial and experimental enzymes showed that a 10

minute 90°C pre-treatment of wine combined with addition of a range of experimental and commercial proteolytic enzymes and incubation at 15°C for 24 hours led to promising losses of protein.

Further trials showed that addition of some enzymes to wine immediately before heating at 90°C for one or two minutes gave better results than preheating the wines and adding the enzymes afterwards.

Two unstable wines, donated by a large commercial company, were processed with two different enzymes on industrial scale heat treatment equipment at the Hight facility of Food Science Australia in June. The enzymes were added to the wines immediately before heating at 90°C for one minute. The wines were rapidly cooled to 18°C after heating.

In general, this combined heat and enzyme treatment reduced the protein levels in both wines to about 40% of the original levels. Heat treatment alone reduced the protein levels to about 75%. The bentonite fining requirement was reduced to approximately 35% in the combined heat and enzyme treated wines, compared to the heat alone treatment that reduced the requirement to approximately 60%, of the bentonite requirement for the untreated wines. In the two wines used for this experiment, this equates to the combined heat and enzyme treatment still requiring between 0.2 and 0.4 g/L bentonite for stabilisation and the heated wines requiring 0.5 g/L.

Sensory evaluation of the wines will be completed in August 2000. Informal assessments indicate that the treatment had



Scientific Research Teams' Reports

little effect on the sensory properties of the wines.

Haze protective mannoproteins

Haze protective mannoproteins or factors (HPFs) were discovered at the Institute in 1991 and are yeast cell wall mannoproteins present in wine (Institute publications #439, 443, 460, 464, and 471). HPFs have the potential to prevent haze formation. Previous work has examined methods to extract HPFs from yeast cells or fermentation media, and two manuscripts describing this work were accepted for publication this year (Institute publication #624 and I.V.S. Dupin, B. McKinnon, M. Bouley, A. Markides, G.P. Jones, P.J. Williams, E.J. Waters, J Agric. Food Chem. in press, 2000). Due to low yields, these methods are not commercially viable. The project is now in the molecular biology stage with the aim to produce yeast strains that overexpress HPFs. Two likely haze protective mannoproteins (Hpf1 and Hpf2) have been isolated and their structural genes identified (HPF1 and HPF2). Patent attorneys have been contacted and a provisional patent is being drafted to protect this intellectual property.

The genes have been cloned and lab yeast strains with the genes deleted have been produced. Lab strains containing an extra copy of the genes fused to a strong promoter have been produced.

Analyses of the mannoproteins produced by deletion and overexpression strains will help determine the contribution of each gene to the production of haze protective

factors by yeast and, thus, allow us to select the best gene for further work.

A bid to augment the HPF project through collaboration within the CRC for Bioproducts was successful. Dr Filomena Pettolino was appointed last year as a postdoctoral fellow to work with Professor Tony Bacic (University of Melbourne) on the physiochemical basis for HPF action. The funds are derived from CRC for Bioproducts and Professor Bacic's group is of outstanding quality.

Dr Pettolino has made good progress in identifying methods to allow for large-scale purification of unstable wine proteins, in preparation for further mechanistic on HPF action. It is hoped that smaller, highly efficient, HPF factors can be identified through these activities.

Studies on random oxidation of bottled wines

Staff: Dr Elizabeth Waters, Dr George Skouroumounis, Mariola Kwiatkowski, Dr Zhong Kui Peng, Dr Leigh Francis

Random postbottling oxidation shows itself in white wines as an obvious browning in a proportion of the bottled wine typically after six to 18 months' storage, and is accompanied by the appearance of oxidised flavour and loss of SO₂ and ascorbate. It has been estimated that the problem costs the industry \$160 million per year in spoiled wine. We have worked in this area since 1995 (Institute publications #528, 534, 550, 551) and, excluding oxygenation of wine

due to poor bottling procedures, have identified variable oxygen permeability through the closure and/or closure/bottle interface as a major cause of the problem. It is possible that other winemaking techniques such as use of ascorbic acid, and upright storage, contribute to random oxidation. This aspect and also an understanding of why closures are permeable have been the focus of the project during the past 12 months.

The inherent permeability of corks to oxygen

A sizeable and ambitious effort by Dr George Skouroumounis to develop a non-intrusive chemical measure for wine oxidation and ingress has not yet been successful. There have, however, been some encouraging experiments.

A water-soluble trap of singlet oxygen, bis-9, 10-anthracene- (4-trimethylphenylammonium) dichloride (BPAA) has been synthesised by Kevin Pardon under George Skouroumounis' supervision. This compound should be appropriate to measure oxygen permeating through closures into model wine, but will be assessed in the next few months. George and Kevin have improved the published synthetic method and currently have more than twenty grams of the material.

As an alternative, a spectrophotometer has been modified to measure browning, the result of oxygen permeation, whilst the wine is in the bottle. This non-destructive method allows individual bottles to be tracked throughout an experiment, and has

also facilitated analyses in the closure trial (see page 37).

A simple modification of a laboratory spectrophotometer has allowed us to rapidly measure wine browning directly in bottles without removal of the closure. This methodology could prove very useful for wineries to establish the development of browning in bottled wine and to establish the extent, if any, of random browning in a large batch of wine.

The effect of ascorbic acid, bottle position and wine type on oxidation

A Riesling and a wooded Chardonnay wine, for an experiment undertaken in collaboration with Southcorp Wines to evaluate the effect of the addition of 100 ppm of ascorbic acid to wines at bottling, bottle storage position and wine type on the extent of browning and oxidation, were bottled in late August 1999. Using the modified spectrophotometer outlined above, browning measurements on every bottle have been taken throughout their nine-month storage period and will continue for at least another year. Other analyses have and will be done at selected times.

The conclusions after nine months storage of the bottled wines were as follows:

The phenomenon of random oxidation (as judged by browning) was not yet clearly evident after nine months storage. Only three bottles out of 775 (0.4%) showed any signs of being 'randomly' browned (the browning in these bottles was higher than the average levels by 3% or more). The very low incidence of 'random' oxidation, as judged by random browning, may be due to the short storage time, and the near ideal storage at the Hickinbotham Roseworthy Wine Science Laboratory under temperature (15-20°C) and humidity controlled conditions. Alternatively, the closures used in this trial may have been particularly non-permeable to oxygen and inserted perfectly. We used reference 2 natural bark corks from two suppliers as well as a synthetic closure and Stelvin screw caps.

For both wines and at all analysis times up to nine months there was greater browning if ascorbic acid had been added to the wine. An example of this is shown with the Riesling wine sealed with either natural cork or Stelvin screw cap in Figure 1 (see previous page). Although the effect of ascorbic acid does not appear as dramatic, the increased degree of browning is consistent with the

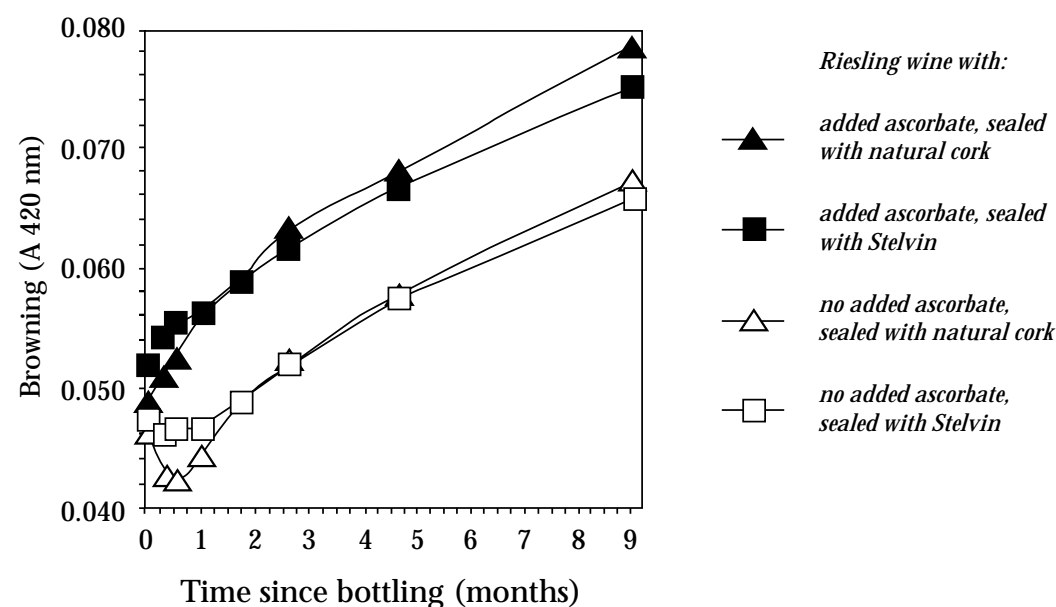


Figure 1. The effect of ascorbic acid addition on the browning of a Riesling wine during storage sealed with either a natural bark closure (natural cork) or with a screw cap (Stelvin). (Note: the degree of browning is very low in any of the samples and not at all commercially unacceptable).





earlier work of Peng *et al.* in laboratory-based experiments (Institute publications #577, 595).

The difference in browning between wines with and without ascorbic acid was small (maximum of 0.012 AU at 420 nm after nine months) and was expected to be difficult to pick by eye. This is to be confirmed.

For both wine types, wines in the bottles sealed with the synthetic closure were consistently more brown than those in bottles sealed with the natural corks. Wines sealed with Stelvin screw caps were the least brown. However, differences in browning between the wines sealed with different closures were very small (maximum of 0.005 AU at 420 nm after nine months) and almost certainly not of practical significance at this stage.

No evidence of pinking has yet been detected.

A sensory difference test after three weeks storage indicated that there was a detectable difference in colour and aroma between the Riesling wines bottled in Stelvin bottles with and without added ascorbic acid.

A statistically significant number of tasters (21 out of 30 responses) picked the Riesling wine without added ascorbic acid as fresher on the palate, when presented with both wines and instructed not to smell the wine.

The panel considered the differences between the two wooded Chardonnay wines to be small when assessing them informally, and under controlled conditions were not able to differentiate the wines on the basis of palate freshness or intensity of oak aroma.

Somewhat surprisingly, the panel was able to pick the wooded Chardonnay without ascorbic acid as having more apparent acidity on the palate than the wine with ascorbic acid.

In all cases, the data from the panel only just reached statistical significance indicating that perceived differences in nose or palate attributes shortly after bottling between these wines with and without ascorbic acid were small, and likely to be of little practical significance.

The aroma impact of ascorbic acid addition was also evaluated after six months of storage. This was tested with wine sealed with Stelvin or the synthetic closure and stored either upright or inverted. The natural closures were not tested. For the Riesling wine, the panel was not able to pick an

Robotic arm with needle

aroma difference between wines with and without addition of ascorbic acid after six months storage. For the Chardonnay, the panel was able to pick the difference with the bottles sealed with Stelvin but not the synthetic closures and the data from the panel only just reached statistical significance. These results indicate that there was no difference in aroma six months after bottling between these wines with and without ascorbic acid. It is mandatory to await the outcomes of longer-term storage before winemaking decisions are based on this experiment.

Readers should note that these conclusions are based on wine stored for a relatively short period of time and that this progress report must be treated as just that. Significant differences between treatments are more likely to be evident after longer storage. The Institute's Industry Services quite frequently see evidence of so-called random oxidation and browning in products and is concerned that this occurs in relatively young wines.

Chemical analysis of industry technical problems including investigations of cork taint

Staff: Dr Mark Sefton, Alan Pollnitz, Dimitra Capone (on extended leave), Yoji Hayasaka, Gayle Baldock

Up to now, this project has had two streams:

- The provision of a problem-solving service to the wine industry which deals with incoming problems *ad hoc*.
- A research program into cork taint, designed to enable the Australian wine industry to undertake a more effective quality control program for cork purchases. Chloroanisole taints in wine, of which cork taint is the most common manifestation, have been the most frequently encountered form of wine taint encountered during the past few years.

As a result of the restructuring of the Institute, the first of these streams has effectively been combined with project AWR 10, which has the same basic objectives (the provision of a service to the wine industry for the solution of major technical problems which require sophisticated instrumentation and applied research). These projects were previously separated according to the expertise of the personnel responsible for their undertaking. Since many problems require a multidisciplinary approach, this division is no longer felt to

be appropriate. Problem solving is now done entirely on a collaborative basis and reporting to industry carried out via the Manager – Industry Services, Mr Peter Godden and the Institute's Oenologist, Mr Adrian Coulter. Accordingly, the results of this work are now given in the report presented by the Industry Services team on page 32.

During the past year, two papers reporting the outcome of our research into cork taint were prepared for publication in the *Australian Journal of Grape and Wine Research*. The first (Institute publication #616) has been published and the second is in the reviewing stage.

As mentioned in our previous annual report, studies confirmed that corks can easily absorb TCA from a contaminated atmosphere without direct contact with the source of contamination, and that such uptake can be rapid in the context of typical transport and storage times. A manuscript on this work has been completed but is awaiting input on the statistical treatment of the data from a consultant statistician before it is submitted to the *Australian Journal of Grape and Wine Research*.

As pointed out in our previous annual report, wines tainted with TCA by contact with contaminated cork closures can be distinguished from those that were contaminated with TCA prior to closure. We further demonstrated that employing the principle of absorption to plastics, methods now exist for at least partially removing chloroanisoles, especially tetrachloroanisole from tainted wines that are commercially unacceptable, so that the wines become commercially acceptable.

A paper titled Absorption of chloroanisoles from wine by corks and by other materials on all aspects of this work has been published in the Australian Journal of Grape and Wine Research (Institute publication #616).

To strengthen the problem solving capacity of the Industry Services team, techniques for measuring potential taint compounds are developed and validated.

A method for analysing wines for naphthalene, which occasionally causes taint in wine, has been fully validated ahead of schedule. 'Problem' wines can now be analysed for this potential contaminant.

Wine grape tannin and colour specification

Staff: Dr Elizabeth Waters, Dr Mark Sefton, Dr Leigh Francis, Dr George Skouroumounis, Dr Zhong Kui Peng, Yoji Hayasaka, Stella Kassara, Mariola Kwiatkowski, Gayle Baldock, Anita Oberholster, Dr Stephané Vidal
Adelaide University staff: Dr Graham Jones, Patrick Iland, Richard Gawel, Robert Asenstorfer, Renata Ristic, Stephanie Lambert, Dr Jim Kennedy, David Lee

This project was initiated by GWRDC as a collaboration between Adelaide University (principally through the projects coordinated by Dr Graham Jones and Mr Pat Iland) and The Australian Wine Research Institute (principally through Dr Liz Water's overall coordination with input from Dr Leigh Francis) in 1996 at the request of industry. It has since been incorporated into Program 1 of the Cooperative Research Centre for Viticulture. An Industry Reference Group composed of thirteen senior red winemakers advises on the project (see list in 1999 Annual Report)

The ambitious objectives of this most challenging project are as follows:

- To gain an understanding of the types of compounds important to red wine colour and mouthfeel and of conditions favouring their presence in wine.
- To determine whether the interaction of proanthocyanidins (tannins) with other grape and wine compounds, especially polysaccharides, modifies their sensory properties.
- To establish viticultural and oenological techniques that enhance wine colour and mouthfeel.

The focus in the previous 12 months has been on wine colour. Since the importance of grape anthocyanins to wine colour is well established and viticultural and oenological treatments to influence the levels of anthocyanins in wine are largely known, most effort has been on the formation of new and more stable wine pigments from the anthocyanins and the influence of tannins and other phenolic compounds on either stabilising wine colour or augmenting colour through copigmentation interactions. Work on compounds important to mouthfeel has also been undertaken in addition to methods required for the project to progress.



In this report, the term 'tannin' will be used in place of the more correct term of proanthocyanidin or polymeric proanthocyanidin. The terms 'pigmented tannins' and 'pigmented polymers' will be used to describe any pigments with high Mr (i.e. greater than 3000 Da). The term 'wine pigment' is not included in the pigmented tannin group. Wine pigments are pigments formed from anthocyanins and low molecular weight wine compounds. Their molecular weight is less than 3000 Da.

Method development

As described below, adding extra seeds to a red must produced wines with more colour, and increased levels of phenolics, including tannins and pigmented polymers. As part of this work, rapid methods to quantify the levels of tannins in seed extracts and in wine have been developed by Dr Zhong Kui Peng as well as sensory methods to describe red wine mouthfeel characteristics by Richard Gawel and Dr Leigh Francis. In addition methods to isolate and characterise wine pigments have been developed by Robert Asenstorfer and Jim Kennedy in Dr Graham Jones' laboratory at Adelaide University, respectively. A paper describing the method to characterise and quantify tannins in seeds has been submitted for publication and the corresponding wine method is currently being written up for publication. These methods developed by Dr Peng are a significant advance on what is currently available because the tannins are clearly separated from other compounds in wine and their identity as polymeric proanthocyanidins (tannins) has been demonstrated rather than inferred.

Using the wine method, Dr Peng has analysed 84 commercial wines of different age. In general, pigmented tannins make up a greater proportion of the pigments in older wine compared to young wine, for example, the level of pigmented tannins as a percentage of total pigments in the 2000, 1999, 1998 and 1997 vintage wines is 16 ± 7 , 38 ± 14 , 54 ± 20 and 69 ± 12 respectively.

The sensory method allows tasters to describe characteristics of a red wine using terminology that all understand and agree upon. A manuscript describing this method has been submitted for publication and is currently under review. The terminology used by the tasters as a mouthfeel wheel has been submitted to the Australian Journal of Grape and Wine Research in order to

reach as many Australian red winemakers as possible. This paper is currently being reviewed.

A paper describing the use of bisulphite to purify anthocyanins has been submitted to Phytochemistry and one describing methods for characterising the structure of grape and wine tannins is being drafted for submission to the Journal of Chromatography. This latter method is an improvement on the current method because most of the conditions have been optimised and the use of a less offensive reagent than commonly used has been well evaluated and recommended.

Wine pigments

Over the last few years, the structures and proposed mechanisms of formation of new wine pigments derived from anthocyanins have been elucidated. In general the reactions involve the formation of new pigments from anthocyanins and other low molecular weight wine components such as acetaldehyde, pyruvate and vinyl phenol. These pigments can be extended further by reactions with monomeric flavanols such as catechin and epicatechin or proanthocyanidin dimers and trimers. These new pigments are more resistant to SO₂ bleaching and more stable under wine conditions.

Robert Asenstorfer, in Dr Graham Jones' laboratory at Adelaide University has demonstrated that the new wine pigments are more resistant to both oxidation and to SO₂ bleaching.

The levels of anthocyanins and new wine pigments in Shiraz wines of varying ages from a single vineyard have been investigated by Robert Asenstorfer. The data clearly show that while the levels of anthocyanins drop to negligible levels in the first few years, the levels of wine pigments decrease relatively slowly and these pigments are still found in wines 35 years of age.

The formation of new wine pigments from anthocyanins depends in part on the chemical properties of the anthocyanins. Robert Asenstorfer's work has indicated that the previous estimations of the protonation and hydration constants of the main anthocyanin, malvidin-3-glucose and its acetate and coumarate form, may be incorrect. These constants determine, in part, the chemical properties of the anthocyanins. Progress in this difficult area of pigment chemistry has been possible because of the use of novel methodologies by Dr Graham Jones

and Robert Asenstorfer such as separation based on electrophoresis.

Revised protonation and hydration constants would mean that the previously proposed pathways for the formation of new pigments may not be the main ones operating in wine and that speculations about oenological treatments likely to influence wine colour through formation of these pigments may be in error. Future trials to examine oenological treatments to enhance the formation of wine pigments will take these new findings into account.

It is widely believed that the anthocyanins also polymerise with tannins from the seeds and skin to form high molecular weight pigmented polymers. These large pigments are believed to stabilise wine colour, and the inclusion of the anthocyanin moiety is thought to reduce the astringency of the tannin.

Anita Oberholster and Leigh Francis have investigated the formation of pigmented polymers in wine. This experiment will strengthen the conclusions from model studies and also test the assertion in the literature that anthocyanins complex with skin and seed tannin and change both their sensory properties (less likely to precipitate with protein, therefore, less astringent) and solubility (more retained in the wine).

A white wine has been made either conventionally (no skin or seed contact), with white skin and seed contact, with white skin and seed contact and added anthocyanins from red grapes, or with red skin and seed contact. Sensory and chemical analyses of the wines were undertaken at six months after bottling.

Fermentation of white crushed grapes to which anthocyanins had been added produced a wine with more tannin, more astringency, more 'grippiness' and more dryness than the wine made without anthocyanin addition. Predictions in the literature that anthocyanins complex with skin and seed tannins to produce 'softer' (less astringent) wines could not be confirmed with this experimental design.

Copigmentation

Another aspect of red wine colour is the phenomenon of copigmentation: other non-pigmented compounds associate with the anthocyanin pigments and augment the colour. Copigmentation makes it difficult to predict the colour of a red wine blend: as the wine is blended, the equilibrium

between the free and copigmented forms of anthocyanins can alter, resulting in less colour than predicted. Anthocyanins acylated with aromatic acids may also be able to self-associate and exhibit greater and more stable colour. A basic understanding of self-association and copigmentation reactions in red wines, the copigments involved and methods to increase the incidence of copigmentation are needed.

Stephanie Lambert and Dr Graham Jones of Adelaide University are undertaking studies on copigmentation. These studies have focussed on the thermodynamics of the interactions and on deriving equilibrium constants that measure the strength of the interactions.

Copigmentation reactions are strongly influenced by pH, with colour enhancement due to copigmentation being greatest at higher pH values. Ethanol decreases the copigmentation interaction.

Of the common simple polyphenolics in wine, quercetin or quercetin-3-glucoside have been shown to form the most stable intermolecular complex with malvidin-3-glucose.

Self-association (i.e. the pigment itself acting as a copigment) of malvidin-3-glucose also appears to be a major cause of colour enhancement. More is definitely better in this case.

Grape seed and skin proanthocyanidins

Grape seeds, together with skins and, to a lesser extent, stalks are a natural source of grape tannins that can be extracted into wines. Amongst industry there are longstanding practices of tasting the grapes in the vineyard to assess the 'tannin ripeness' of the skins and seeds. This empirically important practice is not accompanied by a scientific understanding of tannin biosynthesis and changes during ripeness. This lack of information is partially the result of a lack of methods to measure tannins. A precise and rapid HPLC method to quantify seed tannins is now available (see above). A thorough investigation of tannin accumulation in seeds and skins and their importance to red wine tannin, pigment and sensory aspects can now begin. Data from this subproject will also strengthen hypotheses being formulated in the wine pigment subproject.

A viticultural trial to investigate the differences between seed physical

Orbital shaking water bath with small scale fermentations



Scientific Research Teams' Reports

characteristics (seed number, size and weight) and seed chemical composition of grapes that develop in shaded canopy conditions versus that of grapes that develop in moderately and well exposed canopy conditions has been established by Pat Iland and Renata Ristic at Adelaide University. Samples from the 1999 season have been collected and experimental wines made. An investigation of the impact of skin tannins on wine pigment development and sensory properties has begun with the commencement of Jim Kennedy (a CRCV-funded postdoctoral fellow) at Adelaide University on March 20. Skin samples from the trial described above have also been collected from the 1999 season.

Linking the astringency and mouthfeel properties of wines to proanthocyanidin and or other polymer fractions in wine

Tannins (proanthocyanidins) are well known for their astringent properties in many foods and beverages and it is well accepted that tannins contribute astringency to red wines. Wine astringency is not a simple sensation and winemakers have a host of terms such as hard tannins, ripe tannins, green tannins, puckery, rough, fine, dusty tannins etc to describe different aspects of astringency. To identify the tannins or other compounds responsible for these different sensations and to have quantitative analytical procedures is the initial aim of this sub project. Data from this subproject will also strengthen hypotheses being formulated in the wine pigment subproject. Dr Stéphane Vidal (a CRCV-funded postdoctoral fellow) commenced work in Dr Véronique Cheynier's laboratory at INRA-IPV in Montpellier on 6 June 2000. He will initially isolate tannin fractions from seeds and skins to bring to Adelaide for sensory assessment. He will then apply these methods, and develop new ones if needed, to isolate tannins from wines for sensory assessment.

Winemaking practices that affect proanthocyanidin levels and sensory properties of wine

Good practice in red winemaking is well established and many of the processes that positively impact on red wine quality are known. There are still, however, areas that could benefit from some detailed focussed investigations. The Industry Reference Group, at their meeting on 11 February

2000, identified the use of micro-oxygenation as a priority for investigation.

- Planning for experiments to be conducted next vintage will begin in the next few months.

Yeast flavour and fermentation activity

Staff: Dr Paul Henschke, Dr Mark Sefton, Dr Leigh Francis, Dr Eveline Bartowsky, Jeff Eglinton, Dr Peter Costello, Holger Gockowiak, Dr Josephine Newton, Dimitra Capone

Collaborator: Professor Graham Fleet, The University of New South Wales

The preferred wine yeast, *Saccharomyces cerevisiae*, is responsible for the fermentation bouquet of wine and can, to various degrees, enhance the varietal character of wines. With the aim to extend the diversity of flavours generated during fermentation, other yeast species that are associated with grapes/musts have been selected and evaluated in the laboratory. Winery trials have also been undertaken over the past two vintages. The focus has been on the two yeasts, *Saccharomyces bayanus* and *Candida stellata*, selected from our previous studies. Various aspects of principally laboratory studies on these yeasts which has arisen from PhD studies of Alison Soden, Bryan Todd and Charoen Charoenchais, the Honours project of Mike Fogarty and project work of Scott McWilliam and Jeff Eglinton, are being prepared for publication in scientific and industry journals.

The manuscript The effect of cofermentation with *Candida stellata* and *Saccharomyces cerevisiae* on the aroma and composition of Chardonnay wine coauthored by A. Soden, I.L. Francis, I. Oakey and P.A. Henschke has been published by the Australian Journal of Grape and Wine Research 6(1): 21-30; 2000 (Institute publication #625). Several findings of this publication have been detailed in previous annual reports.

In addition to the main points outlined in Institute publication #625, a further publication #614 disclosed the fermentation and wine aroma characteristics of selected non-*Saccharomyces* yeasts, including *Candida stellata*, *C. krusei* and *Torulaspora delbreuckii*, with Chardonnay. These non-*Saccharomyces* yeasts generally failed to complete fermentation, and *Candida stellata* strains

were found to preferentially ferment fructose. Since fructose is present in many incomplete fermentations, *C. stellata* may have a role in ensuring the complete fermentation of sugar. This is consistent with suggestions by Swiss microbiologist, Dr Jörg Gaffner.

The manuscript *Promotion of autolysis through the interaction of killer and sensitive yeasts: potential application in sparkling wine production* by B.E.N. Todd, G.H. Fleet and P.A. Henschke has been published by the *American Journal for Enology and Viticulture* 51 (1): 65-72; 2000.

The main points of the publication are:

Changes in yeast population and extracellular protein concentration were measured in two commercial sparkling wines over 18 months and showed a short growth phase of about two weeks followed by a prolonged death phase of the yeast which could contribute to a delay in the onset of autolysis.

Protein content of the sparkling wine increased during the yeast growth phase, and a second prolonged phase of protein accumulation followed after 99.9% of the cells had died, marking cell autolysis.

Using protein accumulation as a marker for autolysis, acceleration of the onset of autolysis could be induced by using a mixed culture of a K₂ killer and a sensitive strain of Saccharomyces cerevisiae in which rapid death of the sensitive yeast occurred.

Production of killer toxin was shown to be responsible for the rapid induction of cell death and autolysis by performing the mixed culture fermentation under conditions that inactivated the toxin, such as adjusting the fermentation medium pH to 6 instead of 3.5.

Mixed culture fermentation with two sensitive strains or two killer strains did not however accelerate cell death or autolysis.

Accelerated cell death and autolysis in media at wine pH and containing 10% ethanol suggests that killer and sensitive interactions of Saccharomyces cerevisiae yeast may be used to shorten the time for sparkling wine production.

Mr Jeffrey Eglinton presented a talk on 'Identification and initial characterisation of two *Saccharomyces bayanus* yeasts which have winemaking potential' to the 5th International Symposium on Cool Climate Viticulture and Oenology held in Melbourne on 16-20 January, 2000. The

manuscript coauthored by J.M. Eglinton, M. Fogarty, S. McWilliam, M.A. de Barros Lopes, S. Rainieri, P.B. Høj and P.A. Henschke will be published in the proceedings of the conference. A poster summarising the salient points of the work was also presented.

The main points of the work have been outlined in the 1999 Annual Report but they are:

- Wines made with *S. bayanus* AWRI 1176 (sb1) and 1375 (sb2) contained more glycerol (ca. 3 g/L), succinic acid (ca. 0.5 g/L), SO₂ (ca. 20 mg/L), and less acetic acid (0.3 g/L) than those made with *S. cerevisiae* AWRI 838 (an isolate of EC1118)
- Quantitative sensory descriptive analysis of Chardonnay wines that were made with *S. bayanus* at 18°C clearly showed differences in their sensory properties (aroma and palate) to that made using *S. cerevisiae*. In particular, the aroma of wines made with AWRI 1375 was more diverse than those made with AWRI 1176 or *S. cerevisiae*, showing some fruity ester characters but also the complexing alternative fruit characters.
- Preliminary analysis of the palate showed that wines made with *S. bayanus* had a lower apparent acidity, increased length and weight ('fuller' and more viscous), and more warmth than those made with *S. cerevisiae*. The complexity in mouthfeel was particularly evident on the middle palate, improving the overall appeal of the wines.
- Laboratory-scale wines made with *S. bayanus* contained more acetaldehyde and less ethyl acetate than wines made with *S. cerevisiae*. The contribution of these compounds to the aroma of these wines remains to be determined.

The manuscript entitled 'The effect of fermentation with Saccharomyces bayanus on the chemical and aroma profile of Chardonnay wine' and coauthored by J.M. Eglinton, S. McWilliam, M. Fogarty, I.L. Francis, M. Kwiatkowski, P.B. Høj and P.A. Henschke has been published in the Australian Grapegrower and Winemaker Annual Technical Issue (438a): 28-30, 32; 2000.

- This paper summarises the results of winery based *Saccharomyces bayanus* fermentation trials made during the 1999 vintage, with an emphasis on the sensory attributes of the wines.

- General review comments concerning the winemaking potential of the cryotolerant *S. bayanus* yeast have been included to provide winemakers with comparison of properties expected with typical *S. cerevisiae* yeast, including potential reduced alcohol yield, and increased glycerol and reduced acetic acid formation.

Development of an expertise in the identification and quantification of key sensory impact compounds generated by individual yeasts

The aim of this collaboration with Drs Mark Sefton and Leigh Francis and their teams is to identify and develop measures for the key impact components formed during fermentation, initially with conventional *S. cerevisiae* strains, and subsequently with novel yeasts, such as *S. bayanus* and non-*Saccharomyces* species. Through this, we will develop an understanding of how these important wine flavour compounds are formed and how they can be modulated. Little information is available on the biochemical basis of many sensory properties of wine.

Initially, work has focused on the common fermentation esters produced by yeast which are an important component of the 'fermentation bouquet' of wine, which defines the vinous character as opposed to the varietal character. A review of the literature on yeast-derived flavour compounds progressed by Dr Josephine Newton has given a good focus on the relevant compounds. At this time, progress is ahead of schedule on the development of stable isotope dilution methods for quantifying these compounds; a variety of deuterium labelled ethyl and acetate esters have been synthesised. Work is on track for adoption of these analytical techniques for selected wine key impact compounds by December 2001—these protocols will be used to assess the role of yeast in the evolution of these compounds during the winemaking process.

Members of the Wine Microbiology Team, Dr Peter Costello and Jeff Eglinton, are currently undergoing training on new instrumentation, GC-AED, to facilitate this work. This technique, which permits the simultaneous multi-elemental analysis of volatile aroma compounds, provides a chemical fingerprint that will assist in the identification of important compounds. When coupled with GC-sniff, important and novel aroma compounds can be readily

selected for further analysis. In this way, a library of key impact compounds will be assembled, and appropriate chemical assays developed.

Industry use of new yeast strains — an evaluation of selected yeasts' performance in an industry setting

During the 1999 vintage, four wineries, including BRL Hardy and Yalumba Wines, performed trials to assess the performance of selected novel flavour complexing yeast under winery conditions. Two strains of *Saccharomyces bayanus* and two strains of *Candida stellata*, identified from previous work, (Institute publication # 614 Soden et al., Institute publication #625 Soden et al. and Eglinton et al. Proceedings of the Fifth International Cool Climate Symposium) were evaluated in either stainless steel fermentors or oak barrels. Oak fermented Chardonnay was the main wine type studied.

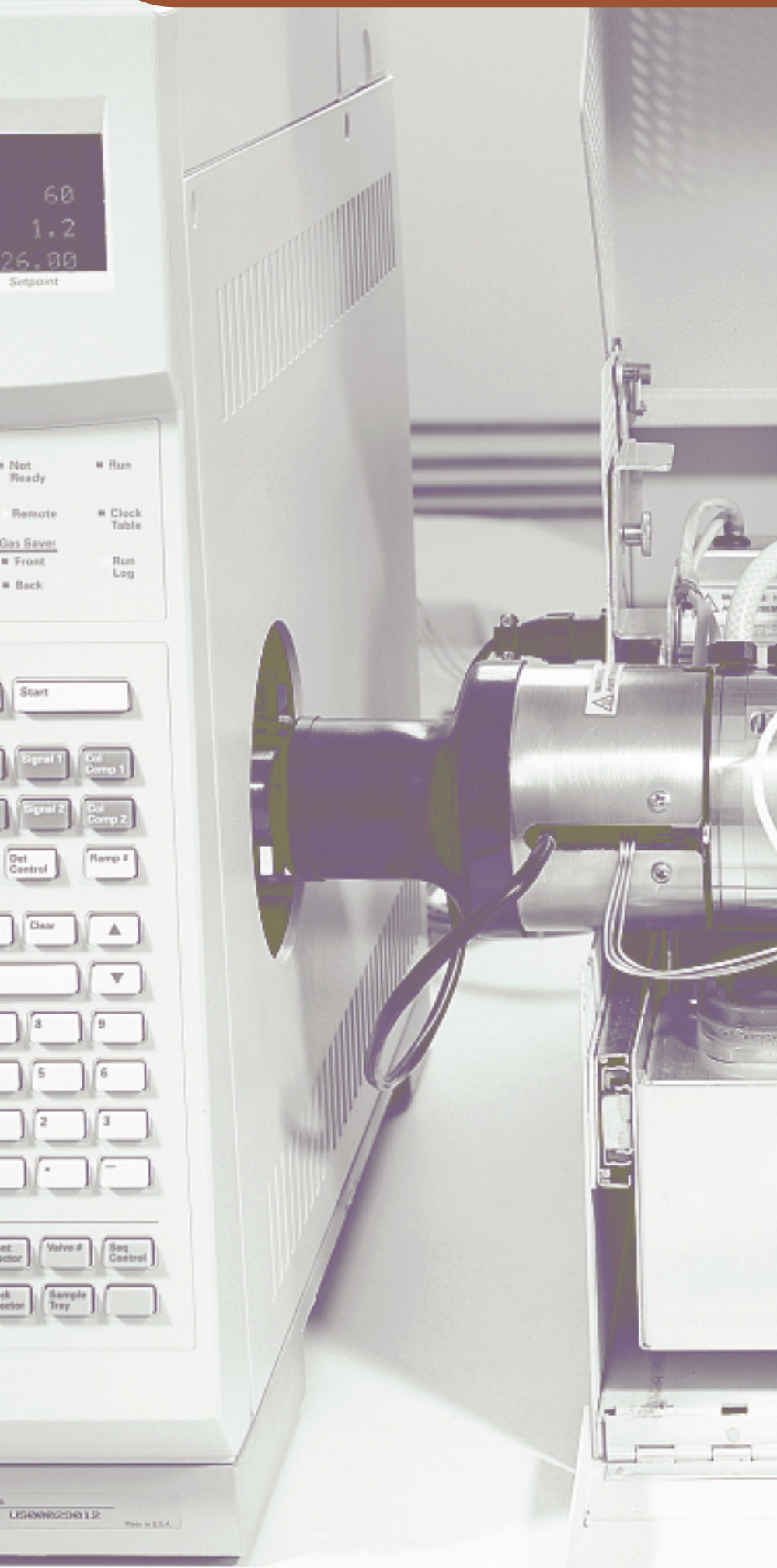
Winemaker comments regarding the potential of these wines for increasing the complexity/diversity of wine aroma and flavour are favourable as detailed below. As the main purpose of these trials was to obtain winemaker comments on the commercial feasibility of these yeasts the trials were limited in nature and consequently only limited chemical analysis and sensory evaluation of the wines has been undertaken.

Further trials were undertaken in the 2000 vintage with the aim to evaluate the compatibility of these yeasts with varieties other than Chardonnay, and included Pinot Noir, Shiraz and Sauvignon Blanc. The chemical and sensory evaluation of these trials should be concluded by February 2001.

Saccharomyces bayanus winery trials

The two strains, AWRI 1176 (sb1) and 1375 (sb2), which have been previously studied in the laboratory as described above, were evaluated under winery conditions. The yeasts showed fermentation characteristics generally similar to *S. cerevisiae* strains, with good growth characteristics but exhibit a tendency to relatively longer fermentation times. This aspect is compatible with barrel fermentation, especially when *sur lie* treatment is also preferred.

Casual sensory appraisal of the wines from all participating wineries showed that these yeasts produced wines with a distinctly different aroma profile from those made with



popular *S. cerevisiae* strains. Furthermore, the wines appeared to show a richer/fuller palate.

Sensory analysis by trained judges showed that wines made with *S. bayanus* by two wineries displayed clearly different aroma properties to those made using *S. cerevisiae*. The *S. bayanus* yeast produced wines that had good fruity/estery characters (floral, estery, pineapple, peach, melon, citrus/lime) with some more complex aromas (honey, nutty, apricot), which may not always be desirable.

As opposed to our laboratory fermentations, none of the wines showed aldehyde-like or oxidised aromas, or H_2S off-odours.

The added aroma diversity in wines made with AWRI 1176 or 1375 was reported by several panel members as positive, with AWRI 1375 producing the most interesting aroma profile because it was intermediate between that of AWRI 1176 and *S. cerevisiae* 838, giving a good balance of the characters that were made by the *S. cerevisiae* and the *S. bayanus* yeasts.

The palate of wines made with AWRI 1176 and 1375 was also different to those made with AWRI 838. Wines made with *S. bayanus* were described as having a lower apparent acidity, increased length (leading to an increased persistence of flavour), a fuller palate weight with an increased viscosity, and more warmth. The overall palate of the wines was richer and fuller, particularly on the middle palate, and this added to their overall appeal.

Wines made with *S. bayanus* AWRI 1176 and 1375 contained more glycerol, succinic acid, SO_2 , and less acetic acid than those made with *S. cerevisiae* AWRI 838. Values for residual sugar, alcohol, malic acid, lactic acid, citric acid, tartaric acid, titratable acidity and pH were generally similar for the three yeasts.

On the basis of these results, additional trials were undertaken during the 2000 vintage. The participating wineries were Yalumba and Montana, who have performed trials with oak fermented Chardonnay, and with Sauvignon Blanc. The Institute values the considerable input from wineries and their willingness to progress joint experiments, whilst subjecting their valuable juices to fermentation trials with yeast that do not always produce the desired outcome.

Atomic emission detector



***Candida stellata* winery trials**

Two strains of *C. stellata*, AWRI 1159 and 861, have been chosen for evaluation under winery conditions. Because of the impaired fermentation properties of these strains, sequentially inoculated fermentations have been performed with a *S. cerevisiae* strain to ensure the complete fermentation of sugar.

Informal assessment of several wines revealed that the influence of the *C. stellata* yeast was subtle and positive. Definition of the Chardonnay fruit character was enhanced and the wine had good palate structure. The greater impact of this yeast species on wine aroma, as was expected from the laboratory trials, was not observed and may have been moderated due to the presence of indigenous yeasts that were not present in the laboratory trials.

On the basis of encouraging winemaker responses the winery-based trials were expanded for vintage 2000. In addition to barrel fermented Chardonnay, trials were undertaken with the red varieties, Pinot Noir and Shiraz. The latter trial, is part of the laboratory scale trial using a winery-prepared must, referred to above. Winemaking has been completed and analysis is in progress.

There has been a surprisingly great deal of interest in this project from winemakers, although it is also fair to say that some industry practitioners believe the yeast to have little influence on the final wine aroma and flavour.

Bacterial metabolites

Little is known about the aroma and flavour metabolism of wine lactic acid bacteria, especially the preferred bacterium, *Oenococcus oeni*. The first step in this investigation is, therefore, to obtain reproducible evidence for significant aroma and flavour changes associated with MLF. Four commercial malolactic fermentation cultures have been used to produce red wines under controlled laboratory conditions for investigating the contribution that bacteria can make to complexing wine flavour. These wines are undergoing chemical analyses.

Bacterial glucosidase activity

Nadia D'Incecco, an Italian PhD student working under the supervision of Drs Bartowsky and Henschke at the Institute, commenced work on two aspects of β -glucosidic activity of malolactic bacteria in December 1999. She is enrolled at the

University of Padova and has a scholarship from the Italian Government.

As part of her PhD studies in Italy, Nadia D'Incecco developed a 'zymogram' technique for yeast β -glucosidase. This technique allows the chromatographic characterisation of enzymes whilst maintaining biological activity on a non-denaturing polyacrylamide gel and detecting specific enzyme activity with appropriate synthetic substrates which form visible/fluorescent products. She has successfully examined a malolactic bacterial β -glucosidase by this technique and showed that this enzyme has properties different from that of *S. cerevisiae*.

As many studies on glucosidase activities in bacteria have involved the use of irrelevant model substrates, the second aspect of the work is to investigate the hydrolysis of an authentic wine glycosidic extract by bacteria in a model wine medium to shed light on the role of bacterial β -glucosidase activity, if any, in flavour development during MLF.

The first round of malolactic fermentations have been completed and GC/MS techniques will be used to see if the bacteria have released any volatile compounds from the glycosylated pool of precursors.

The project has been strengthened by the recruitment of a new PhD student, Kate Howell. Kate was awarded an APRA scholarship which is being administered through the University of New South Wales; Professor Graham Fleet, Dr Paul Henschke and Dr Eveline Bartowsky are her supervisors.

Malolactic fermentation and wine flavour

Correlation of diacetyl content with buttery attributes of commercial wines

In order to test the association of the 'buttery' attribute of wine with the concentration of putative key impact compound, diacetyl, 93 commercial wines are being subjected to chemical and sensory analysis. The wines (Chardonnay, Shiraz and Cabernet Sauvignon) include those for which the diacetyl content was previously measured by the method of Hayasaka and Bartowsky (Institute publication #584) and reported (Institute publication #546). The latter wines have been cellared for approximately 36 months at 12-15°C for a second round of analysis to

establish the stability of diacetyl in bottled aged wine.

The sensory evaluation of 93 wines (36 white and 57 red wines) has been completed by Drs Bartowsky and Francis with technical assistance from Jenny Bellon over a period of six weeks. A panel comprising 18 Institute staff members have rated each wine for the 'buttery' diacetyl character on a scale of 0 to 9.

- The preliminary analyses of data indicates, perhaps not surprisingly, that the perception of diacetyl as a 'buttery' component very much depends on the individual wine in which it is presented. In some cases a wine with relatively high concentration of diacetyl was not rated as high on the buttery scale as a wine with a lower diacetyl content.
- The analyses of wines retained for approximately three years showed that diacetyl was relatively stable in the bottle.

Diacetyl formation by commercial MLF bacteria in wine

Red wine trial

Amongst the factors reported to affect the diacetyl content of wine is the ML bacterial strain used (Institute publication #637). However, no comparative information on bacterial strains is available to winemakers. This project aims to systematically screen popular commercial strains under similar conditions in a red and white wine to characterise these strains so that winemakers can choose the most appropriate strain for manipulating this aroma attribute.

As commercially produced wines sometimes fail to support good MLF, several candidate wines were screened by a pilot MLF experiment. From this trial a Cabernet Sauvignon, 1998, wine (pH 3.5; alcohol, 12.5% vol., malic acid, 2.5 g/L) was selected. Currently, many commercial malolactic bacterial starter cultures are composed of a mixture of strains (currently, six of ten preparations are mixed to enhance performance security), however, it is difficult to interpret the results of mixed culture fermentation due to the different growth responses by each strain in a wine. Therefore, only the four single strain cultures, Chr. Hansen's Viniflora, Lallemand EQ54, Lallemand OSU and Condimenta/Lallemand Bitec/Viacell proVino, are being tested. Biochemical



Scientific Research Teams' Reports

analysis of the wines has focused on diacetyl and organic acids metabolism.

The four cultures showed similar growth and fermentation kinetics in this wine, with a MLF time of approximately three weeks. Each strain also showed a similar pattern of diacetyl and organic acids metabolism. Diacetyl peaked at 8-10 mg/L 3-5 days after the consumption of malic acid and when approx. 75% of the citric acid had been metabolised. After the complete removal of citric acid, diacetyl had fallen to approximately 50% of the peak value. Limited DNA-based diagnostics conclusively revealed that three of the strains were genetically different.

These results suggest that different commercial (selected) malolactic bacteria cultures (at least for those which have been tested in this trial) may have less intrinsic differences in diacetyl metabolism when compared to the greater influences exerted by wine physico-chemical factors (pH, redox potential, alcohol concentration etc.).

Most importantly, these data confirm the transient nature of diacetyl and therefore the importance of the timing of wine stabilisation (e.g. sulfite addition) for controlling the desired level of diacetyl in wine.

A paper entitled 'Management of Malolactic Fermentation for Diacetyl' has been prepared by Eveline Bartowsky and Paul Henschke for the July Technical Issue of the *Australian Grapegrower and Winemaker* (438a, 58-67, 2000)

The main components of this large paper are:

- A table outlining the different winemaking factors which can influence the final wine diacetyl concentration include: ML bacteria strain, inoculation rate, fermentation temperature, pH, citric acid concentration, residual sugar, SO₂, redox potential, and presence of yeast lees.
- Examples illustrating the different winemaking factors affecting diacetyl concentration drawn from our own data as well as published work.
- Discussion on how to combine various factors to influence the final wine diacetyl content.

Interaction between wine yeast and lactic acid bacteria

The aim of the current work is to devise a test system for detecting stimulatory and inhibitory interactions between wine

fermentation yeast and ML bacterium as such interactions have important consequences for the MLF as outlined in previous annual reports. Verification of the test system results with grape juices will allow progress to the second aim, which is to systematically test popular wine yeasts and ML bacteria so that winemakers can choose appropriate combinations for enhancing or preventing MLF. This work, which is being undertaken principally by Dr Peter Costello as a collaboration with Dr Andrew Markides of Adelaide University has been technically demanding work but is now deemed to progress well.

Four *S. cerevisiae* wine yeasts, selected for their apparent differential effects on the performance of ML bacteria (selected on the basis of previous screening experiments, which have been reported in previous annual reports) have been used to produce model wines by the fermentation of a chemically defined medium which resembles the nutrient composition of grape juice. The performance of a selected ML bacterial culture in the four wines was evaluated by growth measurements and malic acid removal.

The results showed that two yeasts produced model wines which stimulated bacterial growth over that of the reference model wine (chemical wine made without yeast). One yeast had a neutral effect on the bacterial culture, that is, growth characteristics resembled those in the reference model wine. That the wine supplement/detoxification treatment did not markedly stimulate growth is difficult to rationalise. The fourth yeast actively killed the bacterial culture, however, the supplement/detoxification treatment reduced the degree of culture death and allowed regrowth after approximately two weeks. Chemical analysis of the wine suggested that SO₂ produced by the fermentation yeast was the cause of culture death. This particular yeast has been noted to vary considerably with respect to the amount of SO₂ produced, but the conditions for this occurrence are not well understood.

The results of this trial suggest that this experimental system may provide valuable practical information on the compatibility between fermentation yeasts and malolactic bacteria; however, this work has only been performed in a laboratory model fermentation system. Work is underway to confirm the validity of the model system

with a grape juice medium. In order to ensure that the grape juice/wine studies are not unduly complicated by interference from non-yeast factors, such as inhibitory substances arising from grape associated fungi/bacteria, or nutrient depletion due to their growth, clean grapes were harvested from three different sites for preparing the wines. The preliminary fermentation trial has been completed.

During the course of the trial, it was noted that the yeast AWRI 838 (an isolate of EC1118), produced an unexpectedly high concentration of SO₂ (>10 mg/L). AWRI 835 (related to AWRI 729), which is a known higher SO₂ producer, also produce elevated SO₂ under similar conditions. Due to our inability to discover conditions which can cause elevated SO₂ production by AWRI 838 in a previous investigation (Institute publication #521), and the implications for our model fermentation studies, and the interest to industry of sporadically high SO₂ production, these studies have been expanded to determine the conditions which cause SO₂ production. This new lead may shed light on why some wines contain higher than expected levels of SO₂ after fermentation. In parallel to this investigation, wines from the preliminary fermentation trial are being challenged with a malolactic bacterium to determine whether these yeasts produce growth inhibitory factors other than SO₂.

Microbiological analysis of industry technical problems

Staff: Dr Paul Henschke, Dr Eveline Bartowsky, Holger Gockowiak, Dr Peter Costello, Adrian Coulter, Peter Valente, Professor Graham Fleet (The University of New South Wales), various winemakers and company oenologists/microbiologists

Rapid molecular identification method for acetic acid bacteria

As reported in the previous annual report, a PCR-based method using primers designed to target a conserved region of the 16S RNA gene has been developed for rapid and reliable identification of acetic acid bacteria. In addition, a PCR-based method (RAPD) for distinguishing and identifying individual strains of acetic acid bacteria has been developed and successfully tested.

The work is being prepared for publication and aspects of the work have been communicated to industry. A talk/

manuscript on 'Microbial spoilage of wine by acetic acid bacteria' was prepared by Eveline Bartowsky and presented to the Fifth International Cool Climate Symposium held in Melbourne on 16-20 January, 2000. The main points of the presentation/paper were:

That a combination of vertical storage of bottles, a small resident population of Acetobacter pasteurianus, ingress of oxygen and the composition of the wine all contributed to the oxidative bacterial mediated spoilage of bottled red wine. The ingress of oxygen appeared to be the critical factor as both spoiled and non-spoiled wines contained resident A. pasteurianus.

Changes in the management of bottled wine transportation and warehouse storage (inverted, instead of upright, bottle orientation) have reduced this spoilage to negligible levels.

The suitability of Near Infrared Spectroscopy for measurement of assimilable nitrogen content of grape juice

Rapid methods for the estimation of yeast assimilable nitrogen (YAN) of grape juices/musts are needed to guide the addition of diammonium phosphate to ferments by winemakers for the purposes of reducing incomplete fermentation and evolution of hydrogen sulfide. This project, which is being pursued in collaboration with staff involved in the project *Analyses of quality parameters in grapes and wine using near infrared spectroscopy*, has the objective to develop and evaluate near infrared spectrometry (NIRS) for estimating the YAN content of grape juice by using free amino nitrogen (FAN), calculated from the amino acid plus ammonium content, and the chemical method based on reaction between the alpha amino group and o-phthalaldehyde/N-acetyl-L-cysteine (OPA/NAC). The latter method has been established at the Institute as a service to industry (Institute publication #587).

Approximately 60 juice samples were included in the set for chemical and NIRS analyses. Scanning was conducted on a NIRSystems 6500 at BRL Hardy, Berri by Dr Bob Damberg. NIRS did not predict the ammonium content, gave equivocal results with FAN, but correlated to a certain degree with total amino acid content (R²=0.61 and SE cross validation= 17.4 mgN/L) of the juice samples. These results are promising but the errors are considered too high.



Left to right: Jenny Bellon, Dr Miguel de Barros Lopes and Nadia D'Incecco

Future more extensive calibration work is required to make use of NIRS for determination of assimilable nitrogen in grape juice.

Species differentiation with chromogenic agar

The rapid indicative identification of yeast and bacteria on quality control agar plates would assist the risk assessment of packaged wine since colony/cell morphology is not a reliable method. Agar plate tests for the presumptive identification of some medically important microorganisms based on enzyme substrate specificity have been developed. The inclusion of a chromogen in the enzyme reaction facilitates a specific colour change which can be used to be diagnostic for certain species. This technology has been evaluated for potential use in the wine industry.

The findings of this work were: (i) three commercial formulations and five individual chromogenic substrates incorporated into routine yeast and bacteria media were tested with representative strains from different wine yeast (32 strains representing 12 species) and bacteria species (17 strains representing 5 species), and (ii) staining differences between various species were relatively small. These results suggest that commercially available chromogenic media tested do not provide a simple and reliable diagnosis of yeast and bacteria associated with wine.

A draft report has been prepared with the main points being:

Staining differences between strains of a given species were relatively small.

Differences in staining patterns between species (e.g. Sacch. cerevisiae versus Sacch. bayanus) were also very small.

From the limited evaluation conducted in this trial, it does appear that a simple and rapid chromogenic medium could be derived to distinguish wine associated yeast (e.g. Saccharomyces versus Candida) or bacterial (e.g. Lactobacillus versus Oenococcus) genera from each other.

No further experimental work is planned.

Selection and improvement of wine yeasts by application of molecular biology

Staff: Dr Miguel de Barros Lopes, Dr Paul Henschke, Jeff Eglinton, Anthony Heinrich, Jenny Bellon, Professor Peter Høj

Construction of a commercial wine strain producing reduced alcohol

As reported in previous annual reports, through overexpression of the *GPD2* gene, we clearly have managed to produce a yeast which produces significantly higher levels of glycerol and small but significant reductions in alcohol content. Unfortunately, as also observed by others, this increased glycerol production is associated with an enhanced production of acetic acid. To alleviate this problem, we previously chose to delete the *ALD7* gene of yeast as we had previously noted that this gene contributes to the generation of acetic acid under fermentation conditions in the presence of oxygen. Re-examination of these results under strictly anaerobic conditions did not lead to a decrease in acetic acid concentration of our ferments.



Scientific Research Teams' Reports

This contrasts with earlier results and could reflect differences in oxygen ingress. Results from other laboratories indicate that the cytosolic Ald6 protein is the main aldehyde dehydrogenase active during anaerobic grape juice fermentation. An *ALD6* deletion strain has been procured and is currently being transformed with the *GPD2* overexpression plasmid. It is hoped that this strain will allow for increased glycerol production and decreased alcohol production whilst also retaining the acetic acid production at acceptable levels.

The strategy chosen to reduce acetic acid in a high glycerol producing strain is to delete a second acetaldehyde gene in the GPD2 yeast. The GPD2 gene has been transformed into a yeast that has had the ALD6 gene deleted. Anaerobic ferments are being done with these modified strains to determine whether deleting the ALD6 gene leads to the desired reduction in acetic acid.

A summary of this work has been published in *Technical Review* and a full manuscript has been accepted for publication in the *Australian Journal for Grape and Wine Research*.

Reduction in hydrogen sulfide formation by application of molecular biology

This research was conducted as part of Catherine Sutherland's PhD work at the Institute and has been described more extensively in a previous annual report. Unfortunately, no conclusive evidence for the success of the approach to reduce the activity of sulfite reductase through a dominant negative strategy has been gathered. Recent data indicates that the most likely reason for the dominant negative strategy not working is that the expression of the mutant subunits was not sufficient. Ms Sutherland finalised her thesis and very positive reports were received from two international authorities in the field of industrial microbiology. Both referees suggested the approach taken had been sensible and that the work certainly contributed valuable new knowledge, some aspects of which were publishable. Ms Sutherland is now undertaking postdoctoral studies in one of Europe's most prestigious Biochemistry Departments at The University of Dundee.

Biological control of indigenous yeasts during fermentation

This project forms part of Nicholas Yap's PhD thesis, and this thesis is expected to be

submitted by 31 July. The quality of Mr Yap's work can be gauged from the recent acceptance of the first refereed manuscript with the title: *The incidence of killer activity of non-Saccharomyces yeasts towards indigenous yeast species of grape must: potential application in wine fermentation*. The paper will appear in the *Journal of Applied Microbiology*.

Use of non-molecular techniques for the improvement of wine yeasts

Rare matings between two diploid lab yeasts have been successful – an important finding for the future rare mating of commercial strains. Mitochondrial mutants of commercial wine yeasts and the winemaking *Sacch. bayanus* strains have been made, providing a selection for rare mating between these yeasts. Hybrids between lab diploid yeasts and commercial wine yeasts and lab diploid yeasts and the winemaking *Sacch. bayanus* strains have been made efficiently using the mitochondrial mutation as a selection. However, achieving rare mating between the commercial wine yeasts and the *Sacch. bayanus* strains has been more difficult. Some potential hybrids have been obtained and their genotypes are being confirmed.

An honours student, Andrew Green, has produced novel hybrids between commercial wine yeasts and strains of Saccharomyces paradoxus using rare mating. The fermentation characteristics of these interspecies hybrids will be tested by fermenting grape juice and chemically defined media.

Identifying genes for wine yeast improvement

Seven DNA fragments that are AFLP amplified in wine strains, but not in the sequenced laboratory yeast SC288, have been cloned in a suitable sequencing vector.

Preliminary sequencing results indicate that novel genes have been identified using this strategy. Whether these novel sequences are widespread in wine strains, and therefore likely to be important for grape juice fermentation, is being determined.

A collaboration with Professor Philip Ganter from Tennessee State University, USA was established to confirm the use of AFLP for studying genetic relatedness in yeast (manuscript accepted for publication).

Instead of using commercial facilities, The Australian Wine Research Institute has, through proceeds generated from our mass

spectrometry facility, contributed to the purchase of an apparatus for two dimensional protein gel electrophoresis in collaboration with The Department of Plant Science, Adelaide University. This will be used for comparing the protein profiles of laboratory strains and commercial wine yeast during fermentation in order to identify proteins that may have essential roles in grape juice fermentation.

Waite Campus Mass Spectrometry Facility

Staff: Yoji Hayasaka, Alan Pollnitz, Gayle Baldock

The four important roles of the Waite Campus Mass Spectrometry Facility are to act 1) as a leader in the application of mass spectrometry to grape and wine research; 2) as an investigator to solve the problems facing the wine industry and individual winemakers, using mass spectrometric techniques; 3) as a collaborator with Adelaide University in research and teaching activities involving mass spectrometry; and 4) as a provider of versatile and advanced mass spectrometric techniques and related expertise to the scientific community including public as well as private institutions.

Usage of the gas chromatograph-mass spectrometer (GC-MS) and electrospray mass spectrometer (ESI-MS) on a time basis by individual groups were 92% and 63% for the Institute; 6% and 14% for Adelaide University; and 2% and 23% for other groups and individuals, respectively. Appropriate financial arrangements for all users are in place to recover the running cost of the Facility. An additional payment is required for non-levy payers and private institutions to recover expenses incurred by staff of the Facility for mass spectrometric analysis and interpretation. Some of the income generated allows upgrades to be made to the facility to the benefit of the regular users of the Facility, the Institute and Adelaide University. In the past year, an autosampler and a diode array detector was purchased for the electrospray mass spectrometer and a new airconditioning unit installed to improve the working environment for employees and instruments alike. The Facility also houses two additional GC/MS instruments for more routine analyses and Alan Pollnitz plays a major role in securing their facile operation.

A new Thermoquest TSQ gas chromatograph mass spectrometer (GC-MS/MS), fully funded by GWRDC, was installed in the Waite Campus Mass Spectrometry Facility in February 2000. This instrument was purchased as a replacement for the 11 year old Finnigan TSQ-70 GC-MS which, until now, had served as our premier research grade instrument in our flavour and problem solving work. The Thermoquest TSQ GC-MS/MS is one of the most advanced, versatile and powerful research grade instruments available today with its capacity to perform MS/MS, electron impact and chemical ionisation, positive and negative mode detection and GC and direct probe inlet. The new instrument will contribute to the current research programs on the identification and fate of trace components which are responsible for the aroma and flavour of grapes and wines. Furthermore the Institute's efforts to investigate juices and wines for potential trace contaminants

will be facilitated. We have further augmented our capacity to analyse samples of grape and wine origin by purchase and installation of a GC with atomic emission detection (GC-AED). Funds for this instrument were secured jointly by the Institute and Dr Graham Jones of Adelaide University as part of a larger application to the Australian Research Council.

The involvement in the research activities of the Institute is one of the most important roles of the Facility. Currently the Facility is participating in ongoing research on the characterisation and quantification of tannins, anthocyanins and grape and wine proteins using ESI/MS, LC-ESI/MS in collaboration with staff and students of the Institute and the Department of Horticulture, Viticulture and Oenology of Adelaide University. It is particularly noted that the research on the differentiation of grape varieties by ESI/MS of juice PR-proteins has been successfully completed as detailed elsewhere in this report.

The role of the Facility to collaborate with the Industry Services on the investigation of suspected taint compounds in juices and wines has become more important due to the ever increasing quality assurance focus of the industry. The Facility conducted more than ten detailed investigations on wines and juices suspected to be contaminated during production, transport or storage.

The analytical approaches for taint investigation are sometimes specialized and complicated due to the search for the trace level of unknown contaminants in the complex matrix of juice, wine or other alcoholic beverages. Investigations typically employ mass spectrometry as a first screen to establish any difference in the extractable compounds between a tainted and untainted sample. If such differences are observed, then both the chemical nature of the taint and its origin will be established where possible.



Sample changer for autotitrator



Industry Services Teams' Reports

General Industry Service team activities

The continuing growth of the Australian wine industry, including the establishment of new wineries and brands on a weekly basis, is generating an increased demand for our services (see Tables 1 and 2). The Institute is convinced these services add value and competitive edge to the industry but also recognise that maintenance of a quality service will require increased resources to maintain its value and responsiveness. In addition to the direct services provided to winemakers on an individual basis, the Industry Services team is conducting a number of large projects as described below and is participating in other projects, such as the NIR projects, as described elsewhere in this report.

Technical problem solving and consultation

Staff: Peter Godden, Mark Gishen, Adrian Coulter, Peter Valente, Ella Robinson

The provision of technical support service to the Australian wine industry (primarily in the form of an advisory service that disseminates a wide range of technical information), and a problem solving and analysis service, represents a significant proportion of the workload of the Industry Services team. In addition, the team manages a major research trial that is examining the technical performance of various types of wine closure, and a major project which is developing a technical reference manual for the Australian wine industry, with associated workshops which will be presented in conjunction with future Institute seminars.

The Industry Services Laboratory analyses several hundred wine samples each year (Table 1), using a wide range of routine and unique analytical techniques, supplemented by detailed sensory evaluation by a panel of experienced tasters. The aim of the service is to offer remedial and preventative advice based on the cumulative problem solving experience of the staff, and the practical winemaking experience of the team Manager and the Oenologist, rather than providing a simple diagnosis of the cause of the problem. Increasingly staff see their role as educational, seeking to disseminate information in a variety of ways in order to

prevent the recurrence of particular types of problem. The Industry Services team also provides technical support to the Institute's Analytical Service, particularly in the maintenance and auditing of the quality management system, contract trial-winemaking services, and the interpretation of analytical results.

Confidentiality is an important aspect of the services provided, and is strictly maintained at all times. This facilitates a frank exchange of information between the Institute and its clients, which in turn allows the maximisation of the knowledge gained from the provision of these services. When a particular problem is considered to be of interest to the wider industry, the results of investigative work are made available through relevant publications, and the Industry Services team contributes regular articles to *Technical Review*. When preparing material for publication, great care is taken to ensure that, under no circumstances is the name of the client, or any possible identifying reference, revealed.

As mentioned above, sensory evaluation is an important analytical and research tool, and is commonly used in problem solving investigations. Since the completion of the Institute's new sensory evaluation laboratory, the team Manager and the Institute's sensory chemist, Dr Leigh Francis, have developed protocols for the continued training and assessment of an enlarged sensory evaluation panel. This ongoing program will use chemical analysis, including GC/MS, to establish the sensory thresholds of members of the sensory panel for a wide range of wine compositional parameters, including taint and wine-fault compounds.

A summary of the number and type of samples received by the Industry Services team over the past three financial years is presented in Table 1. The total number of samples received during the year shows a 13% increase on the previous year, which is a reversal of the recent downward trend in sample numbers. While the increased sample numbers may well be attributable to the difficult conditions experienced over much of Australia in the last two vintages, combined with the general growth in the industry, the sharp increase in the number of samples received in the combined and related areas of hazes and deposits and microbiological instabilities is,



Left to right: Ella Robinson and Peter Godden

nevertheless, disappointing. As noted in the previous year's annual report of the Institute, this area is the first to have been addressed by a new GWRDC-funded project, the *Targeted training of wine industry personnel: compilation of a technical reference manual and development of associated workshops*. Work on this project has focussed on a practical trouble-shooting guide, which is designed to enable wineries to become more self sufficient in the investigation of problem wine samples. A substantial amount of information has been prepared for posting onto the Institute's web-site, and Industry Services staff will deliver the first workshops related to this project, in November and December 2000.

As with previous years, the number of wine samples received with hazes or deposits due to microbiological instability, which have a high pH level, a low concentration of sulfur dioxide, and in some cases incomplete primary or malolactic fermentations, is of continued concern. In addition, these wines have commonly been subjected to a minimal level of filtration.

It is pleasing to note that the number of samples submitted with closure-related problems other than taints has continued to decline. However, the number of major cases of wines with a high incidence of taint, particularly TCA taint, related to the use of natural cork closures, continues at a higher than desirable level, and emphasises the importance of wine companies continuing to screen batches of cork before use.

The types of investigations recorded in Table 1 as 'other investigative analyses' are varied, and some particularly interesting

and unusual cases have been investigated this year.

- Two separate cases were investigated of deposits which appeared to be 'scroll' or needle-like during microscopic examination. These deposits were found to have originated from imported wine bottles, which had become 'weathered' due to high humidity immediately after manufacture. Under these conditions an unstable silicate layer is formed on the inside of the bottle, and this layer can become detached when the bottle is filled with wine. A full report on this investigation, including a simple test for the detection of weathering, was written for the December issue of *Technical Review*.
- Two investigations concluded that deposits found in bottled wine had, in part, been caused by the use of common wine fining agents. One case resulted in calcium tartrate instability, following fining with skim milk powder which was found to contain a high concentration of calcium, and the other was a protein/phenolic complex which consisted of phenolic material suspected to have been extracted from the corks, and protein from isinglass fining.
- Three cases of oxidative pinking were investigated, two of which were in bottled wine. In each case, the wineries concerned had never experienced a pinking problem before in wines made from fruit from the same vineyards as those which were being investigated. It was ascertained that, in each case, the only change in winemaking procedures



Industry Services Teams' Reports

which had occurred was the elimination of the use of ascorbic acid. In one of the cases relating to bottled wine, the pinking was of random intensity between bottles, presumably due to varying levels of dissolved oxygen in the wine. It is possible that the wineries concerned eliminated the use of ascorbic acid following recent Institute publications, which reported on trials which examined the long-term pro-oxidative effect of ascorbic acid (Institute publications #577 and 595). However, it should be noted that the conclusions to be drawn from the Institute's findings are not that the use of ascorbic acid needs to be eliminated. Rather, everything else being equal, the use of ascorbic acid requires a higher concentration of SO₂ to be present in the wine, if long-term browning is to be avoided.

- quercetin di-hydrate was identified as the major component of fibrous deposits which were found in two different bottled wines.
- a Riesling wine, which was received for investigation of an apparently unrelated problem, was noted during routine sensory evaluation to display a 4-ethylphenol character, the major spoilage compound formed by *Brettanomyces* yeast. This type of spoilage is poorly documented in white wine, and is almost exclusively associated with red wines. GC/MS analysis was carried out, and the wine was found to contain 2050 micrograms per litre (parts per billion) of 4-ethylphenol. This would be considered a very high concentration

even for a red wine, based on other samples analysed at the Institute (Institute publication #623 Pollnitz, A., et al, Journal of Chromatography A, 874, 2000, Quantitative analysis of 4-ethylphenol and 4-ethylguaiacol in red wine).

- a red wine was received for the investigation of an apparent failure of filtration equipment, while the wine was being contract-bottled. It was reported that some of the bottles contained a deposit, while others from another part of the bottling run, did not. Investigation revealed that all bottles contained some sort of deposit, but that the nature of the deposit varied markedly between various bottles, which was considered unusual. Sensory evaluation by Industry Services staff indicated that there were also marked sensory differences between bottles, and further chemical analysis confirmed that, in fact, the bottles contained two different wines.

Winemaking consultation

The Industry Services team provides a winemaking consultancy service principally through the Manager, Peter Godden, a qualified and experienced winemaker, Adrian Coulter, who completed the Graduate Diploma in Oenology at The University of Adelaide during the reporting year, and the Quality Liaison Manager, Mark Gishen.

Most queries are technical in nature and arise from Australian winemakers. However, many queries are also received from wine industry suppliers and Government bodies, as well as a relatively

small number from the general public and secondary and tertiary students. Where appropriate, the query is answered over the telephone, by facsimile or e-mail, and Industry Services staff supply approximately 500 technical papers or other pieces of relevant literature to callers each year. Similarly, the support facilities provided by research and library staff are important in supplying relevant information to callers, and the analytical capacity of the Industry Services Laboratory plays an important role in responding to many of these enquiries. In addition, all of the investigations relating to the samples recorded in Table 1 result in a full written report being prepared for the client. These reports contain technical information relating to the problem being investigated and are written in a way which seeks to explain the underlying causes of the problems encountered, and provide advice on how to prevent them re-occurring. The reports are often accompanied by a number of technical references relating to the area of investigation.

The Institute often acts as a referral service, increasingly for Government bodies, wine industry suppliers and wine journalists, having links to Australian and international wine research and political bodies. The vast store of information, both formal (in the John Fornachon Memorial Library) and informal, is a valuable resource to the wider industry.

A summary of the enquiries received by Peter Godden, Adrian Coulter, and Mark Gishen during 1999/2000 is presented in Table 2.

Table 1. Summary of the number and type of samples submitted to the Industry Services team for problem solving during the past three years

Samples received	1997/98	1998/99	1999/00
Identification of hazes and deposits	80	79	114
Microbiological investigations	88	48	85
Sensory assessments	74	52	29
Taint problems	133	117	186
Other investigative analyses	203	245	252
Closure-related investigations	266	154	122
Total	844	695	788

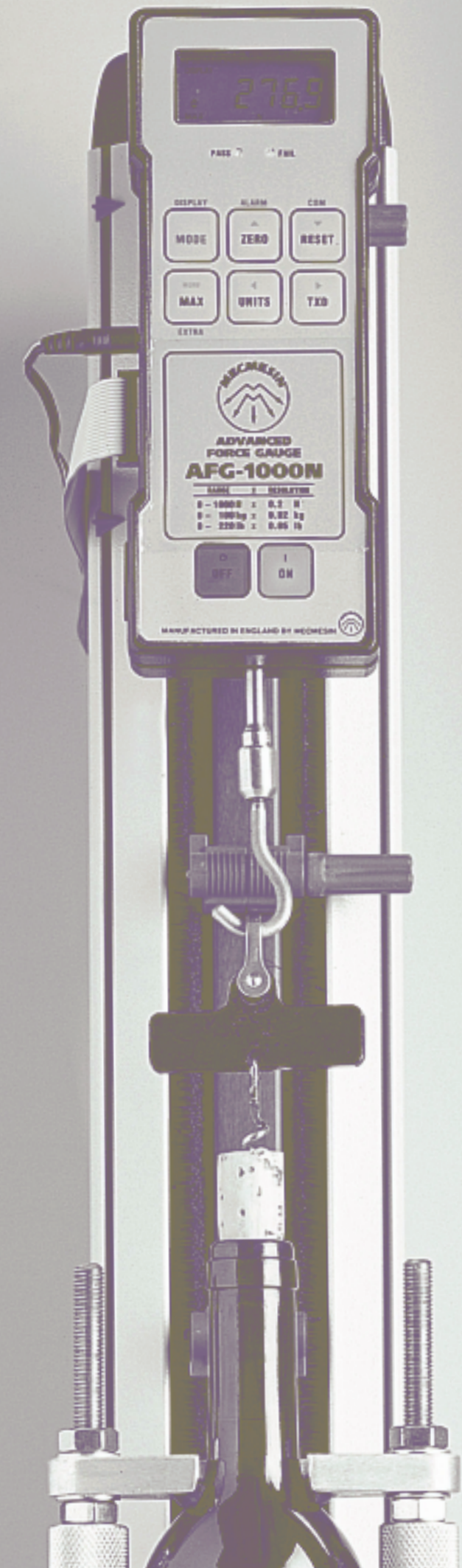
Table 2. Enquiries received by advisory staff during the past three years

	1997/98	1998/99	1999/00
Wineries	853	980	1152
Government organisations	114	70	99
Other	396	419	413
Students	33	35	36
Total	1396	1504	1700

The Consultation and Investigative and Advisory Services are supported by vineyard and winery visits and seminar tours to all major wine growing regions, generally organised in conjunction with local vignerons' associations. The Institute aims to visit each major Australian viticultural region through such formal visits and tours every second, or in some cases third year, as the number of wine growing regions continues to increase. Routine shorter visits by key staff are also made, as opportunities arise—frequently in conjunction with industry events such as capital city wine shows, and seminars held by other industry bodies.

A formal 'Roadshow' visit was made to NSW and the ACT in August and September 1999. Six senior staff presented four full-day seminars, including a seminar in Canberra for the first time. Each seminar consisted of a minimum of twelve presentations, which focussed on current areas of Institute research, or topical issues in the wine industry. Each regional winemakers' association was asked to select the presentations to be made at their seminar, from a list of approximately 40 areas of current Institute activity, in order that each seminar be closely tailored to the interests of the audience in each region. This visit concluded a round of seminars in every major wine growing region of Australia in the previous two years, 22 seminars in total. A full list of industry visits and seminar events in which Industry Services staff participated during the year is provided in Appendix 1.

The twelfth Advanced Wine Assessment Course was held in July, and the thirteenth in December, giving another sixty participants the opportunity to develop and test their sensory evaluation performance. An evaluation of the feedback received from participants over several Courses has demonstrated a change in the profile of participants, and in some cases, their primary motivations for attending, and expectations of the Course. As a result of this feedback, the December Course was expanded to include 44 hours of activities over four days, as opposed to the previous three-day format. Fifteen leading wine show judges, journalists and winemakers, assisted in the presentation of the Course. The demand for the Course continues to be strong and it indicates the need for practical training of industry personnel away from their individual work



Cork extraction test



Industry Services Teams' Reports

environment. As in the past, Associate Judges for the 1999 Adelaide Wine Show were selected from the most successful recent participants in the Course, and other shows have expressed interest using Course results as part of their selection criteria for new Associate Judges. The Course continues to attract interest from the production, marketing, sales and educational arms of the industry, from all States of Australia, New Zealand, and the United Kingdom.

Evaluation of new analytical techniques and of processing aids for winemaking

Staff: Peter Godden, Adrian Coulter, Mark Gishen, Peter Valente, Ella Robinson

The Industry Services Laboratory maintains a GWRDC-funded project for the improvement and development of methods of wine analysis, and the evaluation of winemaking processing aids and additives. The evaluations take one of two forms: the relative performance of commercially available products, and the evaluation of new materials marketed to the industry. Analytical support is provided to the Australian Wine and Brandy Corporation, and the industry as a whole, through the coordination and conduct of surveys of aspects of wine composition. Industry Services staff also provide advice to the Institute's Analytical Service on the development of research protocols, which relate to trials being conducted and analysed on a fee for service basis for various wine companies and industry suppliers.

The major product evaluation activities conducted this year, relate to a wide-ranging trial which is examining the technical performance of various types of wine closure (see Institute publication #593). The trial bottling was conducted under commercial conditions in May 1999, and comprises 14 types of closure, namely: two grades of natural cork; two different types of cork closure which contain a synthetic component; three closures manufactured using different extrusion processes; a closure manufactured from expanded styrene, plus other monomers; five moulded closures manufactured by different processes or from different materials; and a roll-on tamper-evident closure. Various aspects of closure performance are being examined,

including those relating to each closure's physical characteristics and extraction from the bottle, chemical analysis of the wine in order to examine apparent sealing performance, and sensory analysis. In addition, Dr Mark Sefton's team will perform chemical analysis using GC/MS, to examine any effect the closures may have on wine aroma and flavour (see below).

As demonstrated by Table 1 (page 34), problems associated with the use of both natural cork or synthetic closures continue to represent a large proportion of the problem solving investigations conducted by the Industry Services team. However, little is known about the efficacy of use of synthetic closures, and little data is available regarding the relative importance of various parameters of closure performance, by which natural cork, currently available synthetic closures and closures which may become available in the future, can be reliably compared. In addition, little is known about the relative importance of other factors which may effect the way in which closures perform over time, such as the level of oxygen dissolved in the wine during bottling, or the relative importance of storing bottled wine upright, as opposed to inverted.

The broad aim of this trial is, therefore, to elucidate the factors described above, by examining a large number of parameters of closure performance for a broad cross section of currently available closure types, on an ongoing basis for up to ten years. From this data, the relative importance of various performance parameters will be determined, and this knowledge will then be applied to the development of testing protocols, by which the future performance of closures can be rapidly and reliably predicted. In addition, the trial will produce data on parameters relating to bottling and storage, which may then be used to elucidate the importance of those parameters.

As the testing at the twelve-month point of the trial was conducted at the very end of the reporting period, there was insufficient time for a full analysis of the data. However, it is possible to report some highlights from the results so far, as follows.

- At six months there were highly significant sensory differences (0.1%) among the closures, in particular for the aroma attributes scored. There were fewer statistical differences among the

closures for the palate assessments, which were carried out separately from the aroma assessments. Two closures were scored substantially differently from the others: both were rated as significantly lower in fruit attributes, with one closure also rated as particularly high in 'musty' and 'TCA' and the other very high in 'oxidised', 'developed' and 'styrene/solvent like' characters. There were also indications of trends of sensory differences among the other 12 closures, where for example the screw cap closure scored either highest or close to the highest of all the closures in the 'citrus', 'pineapple', 'tropical' and 'overall aroma intensity' attributes, and conversely lowest in attributes such as 'oxidised' and 'developed'. Other closures varied in the levels of these attributes, indicating that the sensory panel could perceive quite subtle intensity differences among the closures.

- A study was made of the data for the set of 12 closures that could be considered to be performing acceptably at the six month period, i.e. not including the results of the two samples showing excessive developed/oxidised or TCA/musty attributes.
- After six months storage, the concentration of free sulfur dioxide, total sulfur dioxide, and ascorbic acid were positively correlated with the aroma and palate scores of the various fruit characters in the wine and 'overall aroma intensity', and negatively correlated with the attribute 'oxidised'. Therefore, the relatively simple measurements of free and total SO₂, and ascorbic acid, appear to act as indicators of the degree of freshness identified in the wine during sensory evaluation.
- at day one, six months and twelve months, there were no significant correlations among the physical parameters of closure performance being examined, ie maximum extraction force, total energy required to extract the closure from the bottle, the torque required to remove the closure from the corkscrew, and the ease of re-insertion of the closure after extraction, with any of the chemical parameters. However, it was found that when the data from the closure that showed high 'oxidised' and 'developed' sensory scores at six months

was included, the closure physical parameters did have a predictive ability—indicating that these parameters may not be of sufficient predictive power to indicate subtle chemical or sensory differences among the closures, but with an extreme case they may be useful.

- The use of optical density at 420 nanometres (OD₄₂₀) has commonly been used as an indicator of the degree of oxidation of wine, being a measurement of relative yellow (brown) colour. However, results of this trial indicate that certain synthetic closures may have the ability to reduce OD₄₂₀ in white wine after bottling, while natural cork may increase OD₄₂₀ at the same stage, by contributing colour to the wine. In this trial, there were no consistent relationships between OD₄₂₀ and free or total sulfur dioxide concentrations, at day one, six months or twelve months, although the closure with the lowest concentration of free SO₂ at twelve months also had the highest reading at OD₄₂₀. Hence, interpretation of OD₄₂₀ measurements may be complex when comparing different closure types, and are certainly more meaningful when comparing bottles sealed with the same closure types.
- There were no major significant differences in measurements of free or total sulfur dioxide for bottles stored upright versus inverted, at six or twelve months. The closure which rated highest for the characters 'oxidised' and 'developed' during sensory evaluation, also had the lowest concentrations of free and total SO₂ when stored both upright and inverted, and the largest difference in mean total SO₂ concentration, being 4.34 mg/L lower when stored upright. While small differences in mean concentrations of free and total SO₂ were apparent with some closures at six and/or twelve months, all of these differences can be accounted for by the experimental error associated with SO₂ analysis, and the differences are not considered to be commercially important.
- There were no consistent relationships between the concentration of dissolved oxygen at bottling (range of 0.6 mg/L to 3.21 mg/L) and concentrations of free and total sulfur dioxide at day one, six months or twelve months.

- Within the major groups of closures included in the trial, i.e. natural cork, cork 'technical', extruded and moulded, there are no consistent trends of increasing or decreasing maximum extraction force, total energy required to extract the closure from the bottle, or the torque required to remove the closure from the corkscrew, apparent in the data from day one, three months, six months and twelve months.
- There were no differences between bottles from each of four corker jaws at six months, for the parameters maximum extraction force, total energy required to extract the closure from the bottle, or the torque required to remove the closure from the corkscrew.
- The concentrations of free and total SO₂ in the wine were 30 and 95 mg/L respectively at bottling. After six months the range of mean concentrations of free SO₂ between the closures, calculated from measurements of ten bottles from each bottling run, was 6 to 24 mg/L with a mean of 19 mg/L, and the range of mean concentrations of total SO₂ was 57 to 91 mg/L with a mean of 83 mg/L. After twelve months the range of mean concentrations of free SO₂ was 4 to 20 mg/L, with a mean of 14 mg/L, and the range of mean concentrations of total SO₂ was 37 to 85 mg/L with a mean of 73 mg/L. In each case the median concentrations were the same, or very similar to, the mean concentrations.

In conjunction with a major Australian wine company, the Industry Services team participated in a sensory and chemical evaluation of a batch of natural corks, which the suppliers maintained had undergone treatment by the DELFIN® (Direct Environmental Load Focussed Inactivation) process. The aim of this trial was to assess the ability of the DELFIN® process to reduce the incidence and magnitude of TCA in wine, which had been stored in contact with the corks. The results of this trial have been communicated in *Technical Review* (April 2000 issue p43-46) which again highlights the extreme care wineries should take in checking the quality of their closure supplies before use.

In conjunction with the Institute's Analytical Service, the following analytical methods have been validated during the year, to the standards required by the

Institute's internal Quality System which is based on NATA requirements and ISO Guide 25: Metals in wine by AAS (validated for new equipment upgrade); Fluazinam in grapes and wine by HPLC; Tebufenozide in grapes and wine by HPLC; Fluazinam in fresh and dried grapes; and determination of ochratoxin A in wine by HPLC. In addition, NATA accreditation was obtained for the methods: Carbendazim in grapes and wine by HPLC; Pyrimethanil in grapes and wine by HPLC; determination of alcohol content of wine by distillation followed by densitometry; and determination of density and specific gravity of wine by densitometry. The following method has been submitted to NATA for accreditation: determination of ochratoxin A in wine by HPLC.

A methods evaluation project conducted in conjunction with the Institute's Analytical Service, related to an evaluation of a Foss Tecator 'WineScan' FT120 FT-IR auto analyser. The instrument can analyse fourteen constituents of wine simultaneously, and the results of previous evaluations conducted in Germany and France have been published recently. The evaluation conducted at the Institute was limited to five constituents: ethanol, glucose/fructose, pH, titratable acidity and volatile acidity. One hundred and seventy three commercial Australian wines were analysed by the Institute's NATA registered reference methods, and then scanned with the WineScan instrument. The WineScan results showed a good correlation with results from the reference methods for ethanol, pH, titratable acidity and volatile acidity (R² range 0.889 to 0.996). For glucose/fructose, the WineScan results were only reliable between five and twenty g/L. The outcomes of this trial have been published (Australian Grapegrower & Winemaker [438a] p75-81, 2000, Institute Publication #636).

Flavour scalping sub-project

Staff: Dr Mark Sefton, Alan Pollnitz, Dimitra Capone

Earlier work from the Institute (Institute publication #616) has demonstrated that both cork and plastic products can absorb volatile components from wine. Any objective assessment of closure performance should, therefore, take into account the inertness or otherwise of the closure in regard to its capacity to scalp wine of its volatile key impact compounds.



Industry Services Teams' Reports

Dr Mark Sefton and his group (Alan Pollnitz, Dimitra Capone and various students) have, therefore, initiated a subproject which is complementary to the main closure trial described above.

New analytical techniques for measuring the concentration of seven fermentation esters, naphthalene, and the grape-derived flavour compounds damascenone, β -ionone, cis and trans-rose oxide, and 1,1,6-trimethyl-1,2-dihydronaphthalene (i.e. TDN the compound imparting a pleasant 'kerosene' /toasted butter character to aged Riesling wine) have been developed and fully validated. The techniques employ stable isotope dilution analysis and are analogous to those used by the Institute to measure oak volatiles and chloroanisoles in wine. These analyses have been developed in order to measure changes in wine composition as a result of flavour scalping and could also be adopted for the commercial analysis of wine.

Deuterium-labelled analogues of 2-isobutyl-3-methoxypyrazine (a compound chiefly responsible for the herbaceous character of many wines from cooler climates), and the monoterpenes geraniol, nerol and linalool (responsible for floral characters in many white wines) have been successfully synthesised. The use of these labelled standards to measure the naturally occurring analogues in wine will be validated.

A large scale bottling trial to measure the capacity of various closures to absorb flavour compounds from bottled wine has been initiated. Both a model wine and a Semillon wine have been spiked with 22 known wine flavour compounds and the wines were then sealed with the closures. The Semillon wine and the closures used are the same as those used in the main closure trial. One hundred and twenty four samples of bottled wine and model wine have been taken after three and six months bottle storage and frozen under nitrogen in glass ampoules. These samples will be analysed once the analytical protocol for 2-isopropyl-3-methoxypyrazine, geraniol, nerol and linalool has been fully validated. Further samples will be analysed after one, two and five years storage.

A parallel study of the capacity of bag-in-box packaging to absorb, from the wines, the 18 compounds for which analytical methods are already fully validated has also been undertaken. More than two thousand analyses were carried out on the samples

taken. The outcomes of this trial will be reported once all the data has been collated and analysed. A preliminary analysis of the data clearly indicates that there is a rapid and early loss of volatile flavour compounds when wine is stored in bag-in-box packaging. The extent of the loss varies widely from compound to compound.

The analytical techniques described above will be used, in the first instance, to monitor flavour absorption from wine. However, accurate measurement of these compounds will also form an important part of future wine flavour studies carried out as part of other Institute projects.

Targeted training of wine industry personnel: compilation of technical reference manual and delivery of complementary workshops

As foreshadowed in last year's annual report, work has commenced on a major new project which aims to produce a flexible and updateable information package on selected technical subjects, which will be delivered to wine grape levy payers via the world wide web (possibly with password protection). In addition, material for use in targeted workshops, which will be delivered to wine industry personnel in conjunction with future Institute seminar visits, is also being developed. A series of six workshops are planned for South Australia and Victoria, in November and December 2000.

The first topic to be addressed by this project is 'Wine instabilities: microbial and physical, and laboratory identification of hazes and deposits in wine.' Combined, these related areas account for many of the wine samples investigated by the Institute's consultation and problem solving service (Table 1, page 34).

A Technical Writer has been appointed, and during the year a technical reference text based on information provided from the Institute's databases has been developed on the subjects of metal-related instabilities, crystalline deposits and glass-related deposits. Work has also commenced on a text relating to microbiological instabilities. In addition, Industry Services staff have developed a comprehensive step-by-step trouble shooting guide for posting on the Institute's website, which has illustrations sourced from routine investigations of

problem wine samples. This guide will gradually be augmented with laboratory methods, AWRI staff papers relating to each subject area, and comprehensive reference lists. It is envisaged that, with the aid of this guide, many winemakers will be able to perform much of the routine problem solving work, which is currently conducted by the Institute.

Trace Analysis Laboratory

Staff: Peter Godden, Greg Ruediger, Kevin Pardon

Work on the CRCV-funded trials examining the fate of agrochemical residues during the winemaking process and subsequent wine storage, was completed during the year, and substantial progress has been made in the analysis of the resulting data and preparation of the results for publication. Due to the completion of these research trials, and in recognition of the nature of the work now conducted, the Trace Analysis Laboratory has ceased to be managed by Industry Services and is now fully incorporated into the Institute's Analytical Service.

Quality Liaison Manager

Staff: Mark Gishen

The major output of the Institute's activities in the provision of advice on quality management techniques to industry remains the *From Grapes to Glass* program, which was published in August 1997 and enhanced with a simple HACCP module in 1999. Industry interest is currently greatest in the HACCP module — a simple program delivered in a one-day course that incorporates an HACCP-type (hazard analysis and critical control point) food safety plan. This module was designed to satisfy the requirements of the proposed (now delayed) changes to the food hygiene regulations, and meet the needs of the smaller scale businesses in the industry. Five courses for the HACCP module were conducted throughout the year, adding 33 more to the growing list of companies having attended. The *From Grapes to Glass* program provides a simple and relatively cheap program that uses a staged approach in the attainment of internationally recognised standards, starting from the Codex HACCP principles and leading to the full ISO 9000 quality management standard.

During the development of the HACCP module of the *From Grapes to Glass* program, a code of good manufacturing practice was drafted in collaboration with the Health and Regulatory Information Manager. With the assistance and input of the Technical sub-committee of the Winemakers' Federation of Australia, the code was finalised and endorsed, before being printed and widely circulated throughout the industry.

Mark Gishen takes primary responsibility for the internal quality management systems of the Analytical Service, overseeing management reviews, documentation, auditing and corrective actions. Reviews conducted throughout the year resulted in improvements in many areas including audit scheduling, and quality control checks in laboratory methods. The Analytical Service continues to participate and excel in both national and international proficiency testing programs for routine wine analysis and for agrochemical residue testing. The Analytical Service continues to manage its quality management system with the aid of the *Paradigm Quality* software package.

Table 3. Summary of information requests during 1999/00

	Wine Industry	Staff	Other	Total	% inc/(dec) (over 98/99)
Information requests	362	203	468	1033	206%
Online database searches	7	29		36	(58%)
Interlibrary loans					
requests sent ¹	25	612		637	14%
requests received ²			73	73	(40%)
Technical Review requests ³	180		20	200	8%
Technical Review articles forwarded ⁴	656		218	874	(18%)
Article requests ⁵	150		50	200	n/a
Articles forwarded ⁶	353		118	471	67%
Number of Institute publications forwarded	363		10	373	143%
Articles photocopied in JFML (March—June 2000)	238	531	100	869	n/a

¹ Staff at the JFML sent a request to another library for an article.
² Requests received by the JFML from other libraries for articles from our collection.
³ Number of requests received for articles published in the Technical Review.
⁴ Number of articles forwarded (usually more than one article is requested).
⁵ Requests received for articles within the JFML library or database.
⁶ Number of articles forwarded, excluding staff publications (usually more than one article is requested).



The collaborative research project evaluating the use of near infrared spectroscopy (NIRS) for the rapid determination of a number of compositional parameters in grapes, must, wine and grape spirit continued and has required input from Mark Gishen. The details of this project are reported elsewhere in the Annual Report.

Provision of technical information

Staff: Rae Blair, Catherine Daniel, Ingrid Oats, Jodi-Anne Slade

The John Fornachon Memorial Library

The John Fornachon Memorial Library holds the largest collection of technical wine literature in Australia. The Library's principal responsibility is to provide technical information to the Australian wine industry and to the researchers of the Institute. The Library is also used extensively by other groups such as students, government bodies and private companies.

Information and document delivery services

The John Fornachon Memorial Library has excellent access to international databases, particularly in the fields of science, technology and medicine. If requested, the Librarian, Catherine Daniel, will carry out online searches on commercial databases on any appropriate topic. The cost of an online search depends on the complexity of the subject and may range from \$50 onwards. Only costs directly incurred in carrying out an online search are passed on to the wine industry client. Alternatively, library staff can provide, free of charge, a report of relevant articles indexed on the Library's in-house databases. The proliferation of free internet and CD-ROM networked databases which publish information, once only accessible through specialist databases, has contributed to recent declines (i.e. 58% decline compared with 1998/99 figures) in requests for specialised online database searches.

Requests for information from Library staff increased dramatically (206%) over 1998/99 figures (see Table 3) and the



Industry Services Teams' Reports

increase can be broken down by category: 160% increase from the Wine Industry; 322% increase from Institute staff; and 209% increase from 'Other.' The overall increase is an indicator of the value and quality of the service, and the effort invested in the communication of its availability. The following statistics help to understand why the increases have occurred.

- A 2% increase in Australian winegrape production is estimated for the 2000 Vintage (1,147,000 tonnes) over 1999 (1,125,840 tonnes) (source: ABS, KPMG). Although this is an insignificant increase, of interest is the shift away from producing 'multi-purpose' grapes to producing specific grapes for varietal wine (e.g. multi-purpose grapes accounted for 30% of total production in 1994, whereas in 2000, they make up 14%). Of note is an increase in production of grape varieties such as Sangiovese (542%), Tarrango (128%), Petit Verdot (126%), Merlot (66%), and Marsanne (22%) (source: 2000 WFA Vintage Report). This shift in focus challenges winemaking and growing practices and management. Additionally, particularly difficult seasonal conditions experienced in all but a few grapegrowing areas also caused challenges for wineries and stimulated an increase in enquiries. Perhaps most importantly, our customer base is

growing with an 8.4% increase in winery numbers recorded in 1998/1999 (1,197) over 1997/1998 (1,104) figures (1999/2000 figures not available) (source: *2000 Australia New Zealand Wine Industry Directory*).

- With regard to demand from Institute staff, 13 new staff and Postgraduate Students commenced duties during the financial year (compared with nine who have left the Institute either permanently or temporarily) and have become active consumers of the service, particularly in the 'start-up' phase of their activities where they often have to familiarise themselves with a new area of knowledge.
- The 'Other' category comprises students, general public, private organisations, international wineries and government. Oenology and viticulture student numbers on the Waite Campus have increased and they continue to be heavy users of the service. Empirical evidence suggests that enquiries received via email have also increased (which is considered to be due to the higher level of visibility the Institute has with its website, and the increasing access to, and use of the internet).

Requests for staff papers also showed a significant increase (143%) which is an indication of the relevance of the research

from the Institute being published, and the clear communication to industry of the information available. Other Library services showed increases in use, with the exception of interlibrary loan requests received (-40%) and requests for articles from *Technical Review* (-18%). The decrease in requests for articles from *Technical Review* is contrasted by a 67% increase in other articles forwarded. This activity will be monitored.

Document delivery

The Library can supply either books or photocopies from its collection or obtain such items for wine industry clients through the interlibrary system. Patents or standards can also be ordered. Electronic ordering and delivery services mean that most interlibrary requests are fulfilled within five days. Charges apply for the supply of some items.

Specialized information services

The library staff continue to be actively involved in the production of specialized information products for the benefit of the wine industry, such as the annual and web-based editions of the *Agrochemicals registered for use in Australian viticulture*, the bimonthly *Technical Review*, and several in-house technical information databases.

Library collection

Seventy monographs and six conference proceedings were added to the Library

collection during 1999/00. The Library subscribes to 54 journals and receives approximately 70 annual reports, journals and newsletters through exchange and donation. The Library also maintains a collection of over 21000 reprints.

Library databases

Apart from a computer-based catalogue of books and journal holdings, the Library has several specialist in-house databases, which index over 32,000 scientific and technical reprint articles; over 2,000 articles on the medical aspects of alcohol consumption; and the bibliographic details of the Library's collection of the European Union wine legislation.

The Librarian, Catherine Daniel, provides reports, either on particular subjects or authors, listing the records retrieved from any of the Library's in-house databases. A summary of the size of the Library's catalogue and information databases is given in Table 4.

Online access project

In March 1999, the Institute submitted to the GWRDC an application to enable the development of a web-based searchable product, that would allow Australian wine and grape levy payers the ability to search through the Institute's databases 24 hours a day from a remote location. This product has been developed and comprises an initial subset of approximately 5,500 records from relevant trade and research publications and proceedings from which permission has been received by the Institute to reproduce published abstracts. This database will be updated on a continuing monthly basis and as negotiations for copyright approvals with numerous industry journals are progressed. This service was officially launched to industry on Friday, 14 July 2000.

To comply with copyright approvals received from the publishers of the various journals, access to this information via the internet is exclusive to Australian wineries and Australian grapegrowers, and available using a secret password and user name previously supplied in writing to the levy payer.

Agrochemicals Grid

As reported elsewhere in this Annual Report, Dr Sally Bell and Catherine Daniel prepared the eleventh edition of the *Agrochemicals registered for use in Australian viticulture*. All levy payers receive a printed



Left to right: Jodi-Anne Slade, Rae Blair and Ingrid Oates

copy of the revised edition automatically, and the web-based edition was revised. The Librarian can, upon request, supply computer-generated printed copies of the updated edition.

Technical Review

Technical Review is received by all Wine levy paying wineries in Australia and, through subscription, by government and other organizations and individuals, both in Australia and overseas. *Technical Review* provides progress reports to the industry on the Institute's research as well as updates on relevant conferences, regulatory amendments and medical issues. *Technical Review's* 'Current Literature' section provides citation details and abstracts of recently published technical and scientific articles. Recipients of *Technical Review* may order articles featured in the 'Current Literature' section via a request form available within each issue. Dr Barbara Hardy AO continues to support the publication of *Technical Review* financially.

Email service

The Email advice and information on technical issues service has proven to be a fast and cost-efficient way of disseminating important technical information to interested members of the Australian wine industry. We are pleased with the response received for this service, as over 218 subscribers have supplied their email addresses to date. Fifteen bulletins, most focussing on changes in agrochemical use, have been issued during the year and are shown in Table 5.

The John Fornachon Memorial Library Endowment Fund

The Institute acts as the Trustee of this fund, which was established in 1969 by donations from the winemakers and friends of the late John Fornachon, the first Director of Research of the Institute. The Library is funded by annual grants from the Institute together with the income generated from investment of the Endowment Fund.

Acknowledgments

The Institute wishes to thank all individuals and companies who contribute to the collection through donations or exchange agreements. The support of the following persons and organizations that have donated books or journals is acknowledged: Australian Bureau of Agriculture and Research Economics, Australian Dried Fruits Corporation, Australian Wine and Brandy Corporation, Australian Wine and Brandy Producers' Association, Commonwealth Scientific and Industrial Research Organization, K.F. Pocock, D.J. McWilliam, Dr B.C. Rankine, Viticultural Publishing Inc., Winemakers' Federation of Australia Incorporated, Yalumba Winery.

Links between viticultural and oenological research

Staff: Alex Sas (until 26/11/99) Dr Sally-Jean Bell (commenced 28/2/00)

Our previous Viticulturist, Alex Sas, has taken up a position as 'Central Viticulturist' with BRL Hardy after four and a half years service with the Institute. Alex is replaced

Table 4. Number of records on the Library's catalogue and information databases

	Number of records
Library catalogue databases	
BOOKFILE: books, conferences and theses	3,198
ARTICLES: scientific papers	32,887
MEDIC: medical papers	2,142
JOURNALS: journals, newsletters, statistics and annual reports	407
Library information databases	
REGS: European Community wine legislation	371
ISYS—full-text retrieval database covering	
United States of America <i>Federal Register</i>	657

Table 5. Email bulletins sent during 1999/2000

Date	Bulletin
2/6/99	Virus detected in grapevines
5/8/99	Agrochemical update
6/9/99	Agrochemical update
10/9/99	Delfin WG agrochemical registration
15/9/99	Agrochemical update
17/9/99	Agrochemical update
20/10/99	Agrochemical update
21/10/99	Grapevine Virus B Fact Sheet
23/12/99	Agrochemical update
21/2/00	Withholding period relaxed on phosphorous acid products
24/3/00	Testing for agrochemicals
27/4/00	Phylloxera outbreak in Strathbogie Ranges
10/5/00	HACCP course rescheduled
7/6/00	Agrochemical update
29/6/00	Copper Sulphate correction



by Dr Sally Bell. The new Viticulturist had, as a first priority, to get up to speed on the agrochemical issues confronting the industry and to prepare a new set of recommendations for agrochemical use.

On 19 May 2000, a meeting was convened with representatives of 16 key wineries to review agrochemical issues of concern to the wine industry, and to set recommendations for agrochemical use in the 2000/2001 season. These recommendations were incorporated into the new edition of *Agrochemicals registered for use in Australian viticulture 2000/2001*. This publication will be placed as an insert in *Technical Review* #127 and the July issue of the *Australian Grapegrower and Winemaker*. Parts of the booklet and website will be included in the Research to Practice™: IPM and Spray Application workshop manuals and in an article in the July edition of *Australian Viticulture*. The information in *Agrochemicals registered for use in Australian viticulture 2000/2001* is also reproduced on the Institute's agrochemicals website, which is updated as information comes to hand.

From July 1999-June 2000 the Viticulturist dealt with 406 enquiries (Table 6), the majority of which were regarding the use of agrochemicals for pest and disease control, the persistence of residues through winemaking and their effects on fermentation, and issues related to maximum residue limits in overseas markets. Many of these enquiries reflected problems related to late season disease control due to unfavourable weather conditions. This prompted an article 'Agrochemicals in the vineyard – use of testing to maintain a clean and green image' for *Technical Review* #125, written by the Manager – Analytical Services and the Viticulturist.

Table 6. Enquiries received July 1999 to June 2000

Enquirer	No. of enquiries
Private individuals	38
Companies*	297
Government Agencies	47
Students	12
Other	12
Total	406

TSQ Mass spectrometer

The extension activities of the Viticulturist are outlined in Appendix 1.

The Viticulturist, in association with Dr Elizabeth Waters, is planning experiments which will complement ongoing oenological research in the Institute's tannin project and in Program 1 of the CRC for Viticulture.

Preparation of information on wine and health issues

Staff: Creina Stockley

This project has been funded since 1990. Ms Creina Stockley, a clinical pharmacologist, assumed this position in 1991 as part of her responsibilities as Health and Regulatory Information Manager. A database of research on the beneficial and detrimental health effects of alcohol and in particular, wine, was established on the internal computer network of the Institute and as part of the John Fornachon Memorial Library. This was facilitated by the subscription to relevant medical and scientific journals, and by the formal and informal exchange of information between complementary organisations, both national and international. During 1999/2000, 67 independent information requests were received on wine and health issues from industry, government and the general public by the Health and Regulatory Information Manager.

Subscription to relevant medical and other journals has continued. The journals have been regularly scanned, the database of research on the health effects of wine has been added to and articles have been prepared for inclusion in the Institute's publication, *Technical Review* and for other Australian wine industry and international alcohol industry newsletters. Articles and other material have also been prepared for the electronic and print media, for example, six articles have been prepared for the bimonthly international publication, *AIM—Alcohol in moderation*, and four articles for the quarterly newsletter of the Australian Society of Wine Education. Seven national radio interviews, one press interview and one television interview were also undertaken related to the research projects coordinated by the Health and Regulatory Information Manager.

Submissions prepared on behalf of the Australian wine industry include:

- *Draft National Drug Strategy—Alcohol Action Plan 1999* of the Federal Department of Health and Family Services;
- *Draft National Drug Strategy 1999–2003: National Alcohol Strategic Plan* of the Federal Department of Health and Family Services;
- *Review of the recommendations regarding responsible drinking* of the National Health and Medical Research Council of Australia;
- *Proposal P167—the review of nutritional labelling: further consultation* of the Australian New Zealand Food Authority; and
- Draft resolutions OENO/SAN/98/110/step 5: importance of research on metabolism of wine compounds producing health effects to the viticulture industry; and OENO/ASP/98/111/step 5: use versus abuse of wine: public policy for the Nutrition and Wine Expert Group of the Office International de la Vigne et du Vin (OIV) (Creina Stockley is a member of the Australian OIV delegation—Nutrition and Health Sub-commission).

Project coordination

Through Creina Stockley, the Institute plays a coordinating and a participating role in three GWRDC-funded research projects on medical aspects of wine consumption. GWRDC pays the Heart Research Institute, The University of Western Australia and CSIRO Health Sciences and Nutrition directly. The Project leaders from these three organisations have agreed to publish on progress of their research here to facilitate dissemination of outcomes to the wine industry.

Reduction of damage to LDL and DNA from oxidative free radicals by the regular and moderate consumption of wine

Principal organisation: CSIRO Health Sciences and Nutrition

Staff: Dr Michael Fenech, Will Greenrod

The majority of the experimental work is conducted by Mr Will Greenrod, a PhD student enrolled at The University of Adelaide with Dr Michael Fenech as principal supervisor and Ms Creina Stockley as co-supervisor.

The aim of the first phase of the project, which was comprised of an *in vitro* and an *in*

vivo study, was to test the hypothesis that regular and moderate consumption of wine protects against oxidative damage to DNA, which is a risk factor for human cancer. For the initial *in vitro* study, samples of whole blood were collected from four healthy male volunteers aged 20–25 years, and supplemented with specific fractions of wine, incubated for 30 min. at 37°C, then challenged with ionising radiation (1.5Gy gamma-rays) or left unchallenged to measure the effects on spontaneous oxidative damage to DNA. The former challenge mimics environmental exposure to carcinogens and the latter represents the baseline or normal conditions of oxidative stress. The samples were then analysed for the extent of chromosome damage using the cytokinesis-block micronucleus assay.

The fractions of wine investigated were: a mixture of the phenolic compounds catechin and caffeic acid; ethanol; glycerol; white wine stripped of phenolic compounds; tartaric acid; and a mixture of catechin, caffeic acid, ethanol, glycerol and tartaric acid. These fractions were added to blood at 2.5% and 10.0% of the concentration found in wine, where 2.5% corresponds to the concentration observed in the body fluids of a 60 kg male after consuming 300 mL of wine.

The results from these *in vitro* studies showed significant reductions in ionising radiation-induced DNA damage in those blood samples that contained catechin and caffeic acid, ethanol, stripped wine and the mixture at both 2.5% and the 10% concentration, although the protective effect was most significant for the 10% concentration. Similar challenges with isolated lymphocytes showed a protective effect of catechin and caffeic acid against hydrogen peroxide-induced DNA damage. Under baseline or normal conditions of oxidative stress, catechin and caffeic acid, ethanol, glycerol, stripped wine and the mixture reduced the incidence of cell death or necrosis. This implies that the wine-derived phenolic compounds, either individually or in combination with the other components of wine, may protect DNA from induced damage.

An acute *in vivo* study was then undertaken in which blood samples were collected from eight healthy male volunteers within two hours after drinking 300 mL of either a 10% alcohol solution in water, de-alcoholised red wine or whole red wine.



Industry Services Teams' Reports

The blood samples were then exposed to ionising radiation. The results from this study showed that ethanol alone increased the level of ionising radiation-induced DNA damage observed in lymphocytes, but that de-alcoholised red wine significantly reduced the observed DNA damage relative to that of the control. Whole red wine gave an intermediate effect with a non-significant trend towards protection, but was significantly more protective than ethanol alone. No significant protective effects were observed, however, for spontaneous DNA damage. Similar *ex vivo* challenge studies have been performed to assess protective effects against hydrogen peroxide.

Thus the results of both the in vitro and in vivo studies imply that the wine-derived phenolic compounds do protect human DNA from induced damage, and may mitigate the DNA damaging effects of ethanol, such that the moderate consumption of wine may be protective for certain cancers.

Grape antioxidant phenolics: absorption and inhibition of lipid peroxidation in humans

Principal organisation:

Department of Medicine, The University of Western Australia

Staff: Associate Professor Kevin Croft, Associate Professor Ian Puddey, Rima Abu-Amsha Caccetta

An initial acute study of 12 male subjects consuming approximately 350 mL of either red wine, de-alcoholised red wine, phenolic-stripped red wine or water over a 30 minute period with food showed that the wine-derived phenolic acids such as caffeic acid are bioavailable, but that such acute consumption does not increase the antioxidant capacity of plasma.

In the subsequent short-term study, 18 male subjects consumed 375 mL of red, de-alcoholised red or white wine daily for two weeks, with a wash-out period of one-week between each beverage. The subjects were all cigarette smokers, chosen specifically as smoking increases significantly oxidative damage and stress. Samples of plasma and urine were collected and analysed for phenolic acid compounds as biomarkers of the absorption of wine-derived phenolic compounds *per se*, and for free and esterified 8-isoprostanes. The latter are oxidation products of arachidonic acid that accumulate in plasma and are excreted in

urine, thus providing a measure of oxidative damage and stress. It has been previously published that the concentration of these oxidation products increases in cigarette smokers and in the presence of ethanol, but decreases in the presence of antioxidants *in vitro* and *in vivo*.

Similar to the initial acute study, following the consumption of the red wine or de-alcoholised wine, the plasma and urine concentration of the analysed phenolic acids increased significantly ($P < 0.001$). As anticipated, the plasma and urine concentration of the free and esterified 8-isoprostanes decreased significantly following the consumption of the dealcoholised red wine, which suggests that the antioxidative wine-derived phenolic compounds decreased the extent of, and hence protect against, *in vivo* oxidative damage. By contrast to dealcoholised wine, the concentration of these oxidation products did not decrease following consumption of red wine with its full alcohol complement.

Hence, while this short-term study fails to confirm the previously published observations that red wine *per se* protects against lipid oxidation, it still suggests that the phenolic compounds are antioxidative and does confirm that the wine-derived phenolic compounds are bioavailable following the consumption of wine. What this short-term study may highlight is the individuality or uniqueness of the cigarette smoking population concerning oxidative stress, but in order to draw such a conclusion further clinical studies are clearly required in both cigarette and non-cigarette smoking population groups.

Potential cardioprotective activities of wine components based on synergistic interaction with vitamin E

Principal Organisation:

Heart Research Developments Pty Ltd.

Staff: Dr Roland Stocker, Ruth O'Halloran

Short-term (one month) administration to apolipoprotein E gene knockout mice of whole wine samples (red, de-alcoholised red and white wines) and fraction 1 derived from them which contained phenolic acids, has been carried out.

So far our results indicate that short term administration of white wine and de-alcoholised red wine have some minor effects on plasma concentrations of

cholesterol, triglycerides and ascorbate, although it is difficult to predict the outcome of these changes on atherosclerosis. In general, the effects of short-term administration of the various wine preparations on the plasma concentrations of lipids or antioxidants appear at best minor. However long-term administration of the same may prove different. Considering these and earlier results, we propose to begin long-term administration of de-alcoholised red wine to apolipoprotein E gene knockout mice ($n = 40$ control & DRW), to test the effect of such treatment on atherosclerosis.

All three subprojects have commenced their third year of funding.

Technical and regulatory support to the Australian wine industry

Staff: Professor Peter Høj, Creina Stockley and Rae Blair

Information requests

One of the activities of the Institute has been to provide legal/regulatory and technical advice and assistance to the Australian wine industry, through the Health and Regulatory Information Manager and the Industry Services team. During 1999/2000, 113 independent information requests on technical and regulatory issues from the government and industry were fielded by the Health and Regulatory Information Manager.

Industry committee membership

Additional support to the industry is derived from the Director's membership on the AWBC Wine Practices Committee, International Trade and Technical Advisory Committee and the WFA Technical Committee. One of the important aspects of the Institute's support of the Australian wine industry is its pivotal role in facilitating the triennial Australian Wine Industry Technical Conference (AWITC), in conjunction with the Australian Society of Viticulture and Oenology. The Director is the Chair, and the Personal Assistant to the Director is the Conference Manager and Treasurer, as well as co-editors (with A.N. Sas and P.F. Hayes) of the Tenth AWITC proceedings. Professor Høj and Ms Blair are also members of the Australian Organising Committee of the 26th World Congress and 81st General Assembly of the Office International de la Vigne et du Vin to be held 11-18 October 2001.

In 1999, the Health and Regulatory Information Manager was a member of the following committees: the AWBC International Trade Advisory Committee, and the Legislative Review Committee; the WFA Technical Committee and Brandy Committee. She is also a member of the Australia New Zealand Food Authority (ANZFA) Expert Working Group on the Review of alcoholic beverages in the Australian Food Standards Code.

Reviews and publications

During 1999/2000, significant efforts, energies and resources have continued to be directed towards the Review of alcoholic beverages in the [Australian] Food Standards Code, in particular the redrafting of Standard P4 for wine, sparkling wine and fortified and Standard P6 for wine products; an additional 13 different position and other papers have been prepared and presented. Considerable effort was directed at the preparation and critique of draft Standard 2.7.4 for wine and wine product of the [joint] Australia New Zealand Food Standards Code.

During 1999/2000, technical information and/or issues that have been reviewed, and discussion or position papers prepared include the *Code of good manufacturing practice for the Australian grape and wine industry*, in conjunction with the Quality Liaison Manager, Ochratoxin A; Silver nitrate; and Tannin and tannin like additives.

Technical information is also disseminated to the Australian wine industry, including all grape and levy payers, by the Institute's bimonthly publication, *Technical Review*, of which the Health and Regulatory Information Manager is editor, and by the Institute's Annual Report edited by the Director and Communication and Publicity Manager.

Australian Wine Industry Technical Conference

Several Institute staff members, namely, Professor Peter Høj, Peter Godden and Rae Blair, serve on the Conference Planning Committee of the Eleventh Australian Wine Industry Technical Conference, and Professor Høj, Mr Godden and Dr Henschke served on the Program Subcommittee. The formal program has been developed and will cover eight two hour sessions from Sunday, 7 October to

Wednesday, 10 October. Some 50-70 workshops are being planned, and the workshop program, being convened by Mr Godden, will run from Sunday, 7 October to Thursday, 11 October. Institute staff will be asked to conduct several workshops and posters will also be prepared for display at the Conference.

This project of technical and regulatory support to the Australian wine industry is ongoing, as technical and regulatory issues are regularly raised by the government or by industry, both in Australia and internationally. Furthermore, these issues often span several years.

Communications and publicity coordination

Staff: Rae Blair

The Communication and Publicity Manager's role is a part-time position for Rae Blair, who is also the Personal Assistant to the Director and Conference Manager of the Eleventh Australian Wine Industry Technical Conference. As Communication and Publicity Manager, she is working to ensure that industry and stakeholder groups clearly comprehend the positioning (value) of the Institute. This positioning is developed in line with the Institute's mission statement and business plan objectives and is guided by our newly developed Communications Policy document. Part of her role is to coordinate the Institute's printed material and other non-technical communications, and to act

as a conduit for media. The Communication and Publicity Manager is also responsible for the performance and output of the John Fornachon Memorial Library. The report of the activities of the Library can be found elsewhere in this Report.

Media

During the year, Institute staff conducted interviews with a variety of Australian media which resulted in the airing or publication of seven radio interviews; 17 articles appearing in national newspapers, magazines and industry publications and six different television programs.

The international media were also interested in the Institute with interviews with staff being conducted with the *London Times*; *BBC Television News* (Frankfurt); freelance journalist (Washington, USA); *L'Express*; *Velo*; *The Economist* (UK); and *Science News* (USA).

Editing

The Communication and Publicity Manager assisted several Institute staff with the editing and/or production of printed material as well assisting in a consulting capacity with the Institute's Advertising Agency.

Selected other activities

Building coordination and liaison activity for the Institute's ca 576 m² eastern extension which was completed in September 1999, and the coordination of the official opening of building.

Left to right: Radka Kalouch, Sandra Lloyd-Davies and Andrea Kemp





Industry Services Teams' Reports



Left to right: Matthew Holdstock and Don Buick

Coordination of the Institute's involvement in the Waite Campus/Agriculture Bureau Open Day held 4 August 1999.

Coordination of presentations given to visitors to the Institute (see report of visitors to the Institute elsewhere in this Report).

Website maintenance

The regular updating of the Institute's website is an ongoing objective for the Communication and Publicity Manager. The Institute staff publications list is updated after publication of every *Technical Review* (every two months). New staff are added where necessary, and 'positions vacant' advertisements are also included. The website is maintained with the assistance of the Computer Systems Officer, Jeff Eglinton.

Analytical Service

Staff: Don Buick (from 14 February 2000), Matthew Holdstock, John Hughes, Greg Ruediger, Gayle Baldock, Amanda Cook, Matthew Cream, Jeremy Hack, Radka Kalouch, Andrea Kemp, Adam Loveys, Kevin Pardon, Randell Taylor, Sandra Lloyd-Davies, Bao Tran and David Boehm

The Analytical Service is a commercial facility, funded by the income generated from fee earning work. It operates as an independent team within the Institute, reporting to the Director, and provides services to the Australian wine industry utilising the practical outcomes of the Institute's published research. Some of its work is performed in conjunction with the

Industry Services team. During the year (14 February 2000), Don Buick was appointed as Manager and he brings considerable experience in analytical chemistry and laboratory management skills to the Analytical Service. Following Sue Week's departure in August 1999, three staff members of the Analytical Service, Matt Holdstock, John Hughes and Greg Ruediger were jointly managing the Service whilst reporting to the Manager – Industry Services on a daily basis and the Director on a weekly basis. Although this arrangement was not sustainable in the long term, it worked well in the short term and the three staff members are to be congratulated on their strong performance.

The services include provision of analyses consistent with the requirements of export certification and support for the quality control activities of winemaking and viticulture which require more sophisticated resources than those commonly found in production laboratories. The Analytical Service conducted over 44,000 individual analyses on wine and grapes in 1999/2000 and offers a wide range of different measurements from routine analyses for sulphur dioxide to complex analyses such as the multi-residue screen for agricultural chemicals. The aim is to provide a range of quality analytical services that are important to industry which can be provided in a reliable, price competitive, accurate and timely manner.

The number of tests performed by the Analytical Service increased by 15% from

the previous year. Other significant increases in test or certificate numbers included testing for AWBC certificates, Japanese Export Certificates which directly influences the numbers of tests for alcohol, glucose+fructose, sulphur dioxide, volatile acidity, and titratable acidity. In addition, malic acid, sensory assessment, trichloroanisole, oak flavours, tebufenozide, pyrimethanil and ethyl carbamate tests have also increased significantly. There was a decline in sample numbers for DNA and in the numbers for the multi residue screen due to deferral of the annual AWBC survey into the next financial year.

Analytical methods for metals, specific gravity and density have been validated following the purchase of an atomic absorption spectrophotometer and a densitometer. Additional services which have been incorporated within the responsibility of the Analytical Service in 1999/2000 include the glycosyl-glucose assay, formerly developed and serviced by the research team, and a method for measurements of colour and phenolics in red grapes. The sensory assessment service has been given a more commercial focus in response to demand from industry, particularly in conjunction with chemical testing for random oxidation or the cause of perceived taints in wine. Opportunities for consulting work utilising a broad range of services and skills including sensory and chemical analysis together with expert interpretation of the results are increasing. This consulting work has often been performed for legal or insurance companies and is used in support of legal action or insurance claims. These clients are not eligible for the free problem solving service provided by Industry Services which is available to levy payers.

Staff have worked closely with the Industry Services team to analyse the 6 month and 12 month samples from the closure trial (see report under Industry Services activities). The analysis of large numbers of free and total sulphur dioxide, dissolved oxygen and OD₄₂₀ were performed with staff working in shifts to complete the work in two to three days of commencement.

Staff have also contributed to the success of the Advanced Wine Assessment Course conducted in 1999 and the planning of the July 2000 course. Activities include the purchasing of wine, setting up the wine for

sensory assessment and recording of the trainees' results for evaluation.

A new fee schedule is nearing completion following a review of all prices to accommodate the Goods and Services Tax (GST) from 1 July 2000 and to incorporate new services into the schedule which was last updated in 1998. Similar changes will be made to the information on the web site located at

(<http://winetitles.com.au/awri/index.html>). The pricing review has identified the need to increase prices for selected services (excluding GST) whilst other services will be reduced in price reflecting improved methods or greater efficiencies.

The Analytical Service continues to hold NATA accreditation for many of its services and is due for re-assessment in the near future. Two new methods have been proposed for accreditation including ochratoxin A and acetic acid by enzymatic assay.

The Trace Analysis Laboratory, operating within the Analytical Service, performs more specialised testing including the multi-residue screen which includes 28 residues, covering over 50 brand name agrochemicals. The analysis is accredited by NATA and is regularly exposed to international proficiency testing. Increasing interest from Australian and overseas clients in a number of specialised services including the trichloroanisole and oak flavour analyses is evident from sample numbers and recent enquiries and is anticipated to provide continuing growth in the coming year.

A new gas chromatograph with a mass selective detector was installed in the Trace Analysis Laboratory during the year and this is now fully operational providing residue testing, TCA and oak flavour analyses. A fluorescence detector for the HPLC was also purchased which has enabled the service to validate a method for Ochratoxin A in wine. A method for fluazinam has also been validated.

A number of residue trials have been performed for chemical companies during the year to establish the levels of residue present or absent in grapes or wine following the application to vines of newly developed agrochemicals. These trials involve testing of grapes, small scale winemaking and analysis following development and validation of methods of analysis. The data produced is used by the chemical companies for further development of agrochemicals and for application to the National Registration Authority for registration approval.

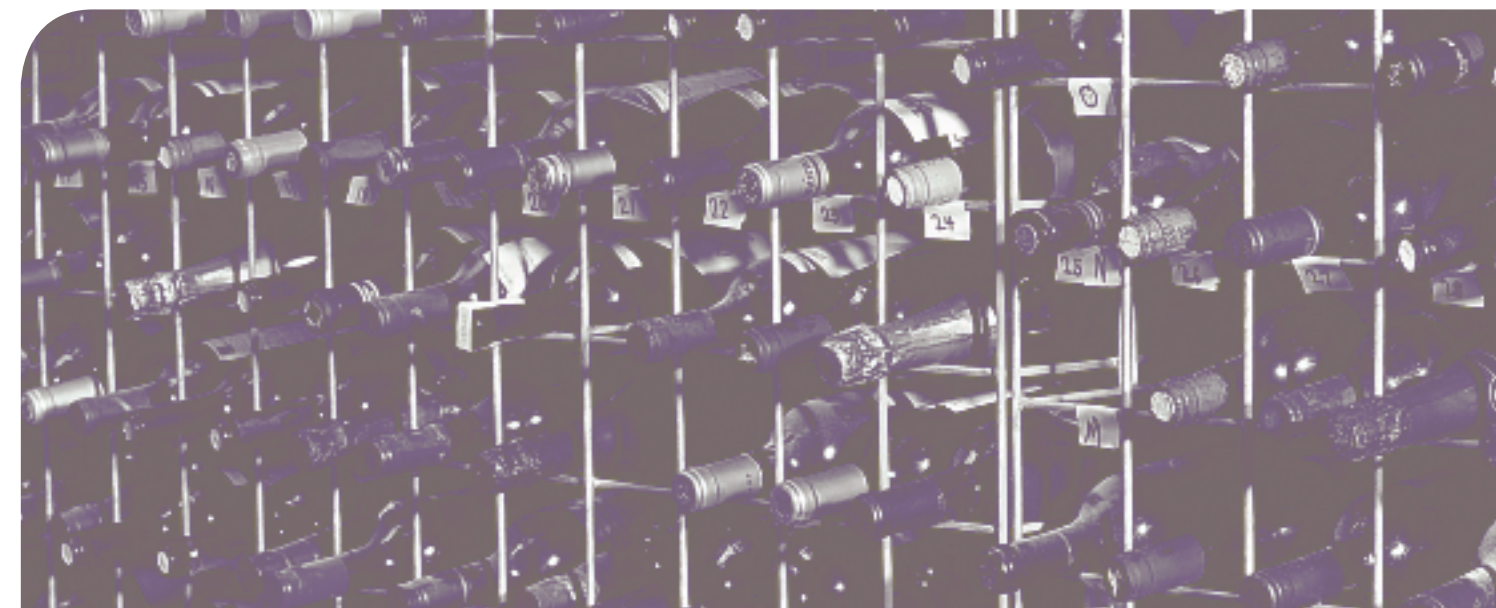
Greg Ruediger, the Trace Analysis Laboratory Supervisor, has completed a Graduate Diploma in Oenology during the year to qualify as a winemaker. Greg's increased expertise and knowledge expands the ability of the Analytical Service to understand the problems facing our clients and to improve our service. Over the next three years, Matt Holdstock, Analytical Service Supervisor-Laboratory, will undertake part-time studies to complete the Graduate Diploma in Oenology. The Analytical Service sees this continual upgrading of personnel skills as a

cornerstone in our ability to include specialised contract work in our portfolio of services.

The policy of continuing to invest in new equipment and making capital repayments over three years from the surplus generated by the Analytical Service has allowed the introduction of new technology and will continue to provide benefits for the Analytical Service based on improvements in the efficiency, capacity, quality and range of services provided.

Following the expansion and upgrading of its administrative and laboratory facilities, including the relocation of the Trace Analysis Laboratory, the Analytical Service staff now enjoy the benefit of excellent facilities and a good working environment.

The Analytical Service advertising strategy includes the placing of advertisements in industry journals and publications, circulation of the fee schedule and maintenance of an informative web-site. For the first time, the Analytical Service will be sponsor of a trophy at the Royal Adelaide Wine Show in September 2000. This will provide exposure to a large number of winemakers and industry representatives. We believe these initiatives will facilitate the continued growth of the Analytical Service for the benefit of the Australian wine industry.





Abridged accounts of the financial report
for year ended 30 June 2000



Left to right: Janet Currie and Narelle D'Costa

Statement by directors

In the opinion of the directors, the abridged accounts of The Australian Wine Research Institute for the year ended 30 June 2000, as set out on pages 48 to 51, are drawn up so as to give a true and fair view of the company's financial position as at 30 June 2000, and its performance for the year then ended, having been extracted from the audited financial reports which are enclosed herewith at the back of the Annual Report or are available upon request.

Signed in accordance with a resolution of the Board of Directors this 30th day of October 2000.

D.J. McWilliam
Chairman

P.B. Høj
Director



ABRIDGED PROFIT AND LOSS STATEMENT
FOR THE YEAR ENDED 30 JUNE 2000

	2000 \$	1999 \$
Operating income		
Grape and Wine Research and Development Corp project funds	3 191 013	2 709 191
Other project funds	409 742	518 986
Analytical Service	1 130 203	926 769
Sundry income	228 177	120 947
Total income	<u>4 959 135</u>	<u>4 275 893</u>
Operating expenses		
Staff	2 961 477	2 486 439
Administration and project costs	1 817 187	1 360 164
	<u>4 778 664</u>	<u>3 846 603</u>
Operating Profit before Interest and Capital Grants	<u>180 471</u>	<u>429 290</u>
Net interest received	<u>67 825</u>	<u>60 752</u>
Operating Profit before Capital Grants	<u>248 296</u>	<u>490 042</u>
Capital grants received:		
Grape & Wine Research and Development Corporation		
Equipment	722 514	129 000
Building	250 000	125 000
Depreciation amortisation and profit/loss on disposal	<u>(338 130)</u>	<u>(275 466)</u>
	<u>634 384</u>	<u>(21 466)</u>
Operating profit	<u>882 680</u>	<u>468 576</u>
Retained profits at the beginning of the financial year	2 505 262	2 036 686
Retained profits at the end of the financial year	<u>3 387 942</u>	<u>2 505 262</u>



Abridged accounts of the financial report
for year ended 30 June 2000

BALANCE SHEET
AS AT 30 JUNE 2000

	2000 \$	1999 \$
Current assets		
Cash on hand and at bank	120 020	228 072
Trade and other receivables	346 413	291 991
Short term deposits	648 000	621 000
Other	25 621	-
Total Current Assets	1 140 054	1 141 063
Non-current assets		
Leasehold buildings	2 102 021	1 459 249
Plant and equipment	1 960 983	1 395 224
The Australian Wine Industry Chair of Oenology	840 000	840 000
Total Non-Current Assets	4 903 004	3 694 473
Total assets	6 043 058	4 835 536
Current liabilities		
Accounts payable	436 328	378 714
Provision for employee entitlements	362 822	274 273
Loan	170 000	-
GWRDC funds not expended	51 877	-
Total Current Liabilities	1 021 027	652 987
Non-current liabilities		
Provision for employee entitlements	64 272	107 470
Total Non-current Liabilities	64 272	107 470
Total liabilities	1 085 299	760 457
Net assets	4 957 759	4 075 079
Accumulated funds		
Retained profits	3 387 942	2 505 262
Asset revaluation reserve	603 067	603 067
Capital reserve	966 750	966 750
Total accumulated funds	4 957 759	4 075 079



THE AUSTRALIAN WINE RESEARCH INSTITUTE
STATEMENT OF CASH FLOWS
FOR THE YEAR ENDED 30 JUNE 2000

	2000 \$	1999 \$
Cash flows from operating activities		
Grants and other income	5 929 599	4 433 588
Interest received	67 825	60 752
Payments to suppliers and employees	(4 701 320)	(3 885 027)
Net cash provided by operating activities	1 296 104	609 313
CASH FLOWS FROM INVESTING ACTIVITIES		
Redemption of (payment for) investments	(27 000)	(41 000)
Payments for building improvements	(684 620)	(422 681)
Payments for plant and equipment	(911 819)	(390 088)
Proceeds from sale of plant and equipment	49 283	69 750
Net cash used in investing activities	(1 574 156)	(784 019)
CASH FLOWS FROM FINANCING ACTIVITIES		
Proceeds from loans	170 000	-
Net cash provided from financing activities	170 000	-
Net increase (decrease) in cash held	(108 052)	(174 706)
Cash at 1 July 1999	228 072	402 778
Cash at 30 June 2000	120 020	228 072

RECONCILIATION OF NET CASH PROVIDED BY
OPERATING ACTIVITIES WITH OPERATING PROFIT

Operating Profit	882 680	468 576
Non-cash flows in operating profit:		
Amortisation and depreciation	338 130	275 466
Profit on the sale of plant and equipment	495	(16 196)
Charges to (reduction in) provisions	45 351	(43 682)
Changes in assets and liabilities:		
(Increase) / Decrease in inventories	(12 000)	
(Increase) / Decrease in receivables and prepayments	(68 043)	(77 468)
Increase / (Decrease) in sundry creditors and accruals	109 491	2 617
Net cash provided by operating activities	1 296 104	609 313



Appendix 1
External seminars, talks and poster papers presented by
Institute staff during 1999/00

External seminars and talks

Author	Title	Organisation/Location	Date
P.B. Høj	From research to practice: should scientists be involved and if so how? Examples from the Australian wine industry	Public lecture at Adelaide University, Adelaide, SA	2 July 1999
P.B. Høj	Stanhope Oration	Annual conference of the Australian Science Teachers' Association, Adelaide, SA	5 July 1999
I.L. Francis	Introduction to the aroma and sensory facility	SASTA conference Hickinbotham Roseworthy Wine Science Laboratory, Waite Campus, Urrbrae, SA	7 July 1999
M.A. de Barros Lopes	Genetic improvement of wine yeasts	Faculty of Science and Agriculture, Charles Sturt University, Wagga Wagga, NSW	16 July 1999
A.N. Sas	Quality issues for viticulture	CRCV Grape Quality Symposium. Charles Sturt University, Wagga Wagga, NSW	17 July 1999
M. Gishen	Integration of HACCP into quality management systems in the wine industry – <i>From grapes to glass</i>	Sixth Australian HACCP Conference, Adelaide, SA	10-12 August 1999
A.N. Sas	Using agrochemicals – selling wine	Department of Natural Resource and Energy, Trawool, Vic	13 August 1999
M.A. de Barros Lopes, S. Rainieri ¹ , P.A. Henschke P.R. Langridge ²	The analysis of genetic variation in yeast using AFLP	IX International Congress of Mycology, Sydney, NSW	16-20 August 1999
P.W. Godden, P.B. Høj, P.A. Henschke, A.N. Sas, E.J. Waters, M. Gishen	Roadshow visits	Regional winemakers' associations Canberra ACT Griffith NSW Mudgee, NSW Hunter Valley, NSW	30 August 1999 31 August 1999 2 September 1999 3 September 1999
R.G. Dambergs ³ , M. Gishen	NIRS in the new millennium: applications for the wine industry — quality assessment of grapes, wines and spirits on the potential applications of NIRS	Winery Engineers' Association annual conference. Griffith NSW	9 September 1999
A.P. Pollnitz, K.H. Pardon, M.A. Sefton	4-ethylphenol and 4-ethylguaiaicol in Australian red wines	Use of oak barrels in winemaking seminar. Australian Society of Viticulture and Oenology, Adelaide, SA	7 October 1999
C.S. Stockley	An update on the Australian wine industry from a research and regulatory perspective	1999 Conference of the International Medical Advisory Group (of brewers), Melbourne Vic	7 October 1999



Author	Title	Organisation/Location	Date
P.B. Høj	Introduction to the new publication, <i>Tasting success – South Australian wine</i>	Hyatt Regency, Adelaide SA	12 October 1999
P.W. Godden	Wine closures – Australian market perceptions, expectations and future	Cork & Seals seminar. Waite Campus, Urrbrae SA	27 October 1999
P.A. Henschke	Indigenous non- <i>Saccharomyces</i> yeasts in winemaking	NZSVO Conference, Auckland, New Zealand	4-5 November 1999
C.S. Stockley	What can Australia learn from other countries? Alcohol-specific strategies	Australian Professional Society for Alcohol and Other Drugs, Canberra ACT	8 November 1999
P.B. Høj, R.E. Day, J. Keniry ⁴ , S. Gerlach ⁵	The Australian Wine Industry – success through innovation	Prime Minister's Science Engineering and Innovation Council, Canberra ACT	26 November 1999
I.L. Francis	Assessment of grape quality	Southcorp Growers' meeting, Karadoc Winery, NSW	26 November 1999
M. Gishen	Potential of NIRS applications in the wine industry	Representatives from Southcorp Wines, The University of Melbourne, Agriculture Victoria, Padthaway Precision and the Bread Research Institute. The University of Melbourne, Parkville Vic	3 December 1999
P.B. Høj	An overview of recent developments of oenological research and its practical implications	Southcorp's Winemakers' Forum. Tanunda, Barossa Valley SA	9 December 1999
P.B. Høj	Chair of session: Flavour development in grapes and wine	Fifth International Symposium on Cool Climate Viticulture and Oenology, Melbourne Vic	16-20 January 2000
P.B. Høj	Recent developments in the application of NIR to grape quality measurements	Fifth International Symposium on Cool Climate Viticulture and Oenology, Melbourne Vic	16-20 January 2000
E.J. Waters	Tannins – with special reference to cool climate wines	Fifth International Symposium on Cool Climate Viticulture and Oenology, Melbourne Vic	16-20 January 2000
P.A. Henschke	Yeast from a can or on the grape?	Fifth International Symposium on Cool Climate Viticulture and Oenology, Melbourne Vic	16-20 January 2000
J.M. Eglinton	Identification and initial characterisation of two <i>Saccharomyces bayanus</i> yeasts which have winemaking potential	Fifth International Symposium on Cool Climate Viticulture and Oenology, Melbourne Vic	16-20 January 2000
E.J. Bartowsky	Microbial spoilage of bottled red wine by acetic acid bacteria	Fifth International Symposium on Cool Climate Viticulture and Oenology. Melbourne Vic	16-20 January 2000



Appendix 1
External seminars, talks and poster papers presented by
Institute staff during 1999/00

Author	Title	Organisation/Location	Date
P.B. Høj	Pre-vintage address	King Valley Grapegrowers' Association at their Pre-Vintage Dinner, King Valley Vic	22 January 2000
I.L. Francis	Grape colour and Grape G-G: practical indicators of wine style?	Unified Grape and Wine Symposium, Sacramento, California, USA	27 January 2000
I.L. Francis	G-G and berry colour measurement	Gallo Winery, Modesto, California, USA	31 January 2000
P.W. Godden	Tasting of Italian wines and presentation on indigenous Italian grape varieties	Adelaide Hills Winemakers' and Grapegrowers' Association. Adelaide Hills, SA	4 February 2000
M. Gishen, D. McLennan ⁶	The G-G assay and NIRS: what are they and what are the applications in the wine industry?	Orange Region Vignerons Association Annual Field Day. Orange Agricultural College Machinery Centre, Orange NSW	5 February 2000
P.B. Høj	Facilitator – Clusters in Science	National Innovation Summit, Melbourne Vic	9-11 February 2000
E.J. Waters	Vineyard management to meet grape quality specifications	Israeli grapegrowers, Plant Research Centre, Waite Campus, Urrbrae SA	11 February 2000
M. Gishen	Options in quality management for grapegrowers	Clare Valley Winegrape Quality Improvement Group, Clare SA	11 February 2000
G.K. Skouroumounis, M.A. Sefton	Mechanisms in the formation of β-Damascenone	Carotenoid Symposium – American Chemical Society, San Francisco, USA	27 March 2000
G.K. Skouroumounis, M.A. Sefton	Mechanisms in the formation of β-Damascenone	E&J Gallo Winery, Modesto, CA, USA	29 March 2000
M. Gishen, P.J. Clancy ¹¹ , I.L. Francis, R.G. Dambergs ³ , R.S. Johnstone ⁷	Development of a robust portable scanning NIRS instrument for quality assessment of grapes in the wine industry	Ninth Australian Near Infrared Spectroscopy Conference, Horsham Vic	5-6 April 2000
E.J. Waters	Program 1: vineyard management to meet grape quality specifications	CRCV Board, Plant Research Centre, Waite Campus, Urrbrae SA	3 May 2000
M.A. Sefton	Cork taint	Margaret River Field Day, Margaret River, WA	5 May 2000
M.A. Sefton	4-ethylphenol	Margaret River Field Day, Margaret River, WA	5 May 2000
S.J. Bell	Environmental factors affecting the flowering process of <i>Vitis vinifera</i>	Coonawarra Grapegrowers' Association, Coonawarra, SA	10 May 2000



Author	Title	Organisation/Location	Date
P.A. Henschke	1. Off-flavours in wine from <i>Dekkera/Brettanomyces</i> yeast and lactic acid bacteria: survey of 4-ethylphenol in Australian wines; 2. Off flavours in wine from <i>Dekkera/Brettanomyces</i> yeast and lactic acid bacteria: mousy N-heterocycles from <i>Dekkera/Brettanomyces</i> and lactic acid bacteria 3. Genetic strategies to reduce H ₂ S formation by yeast	Department of Enology and Viticulture, University of California, Davis, USA	16 May 2000
P.A. Henschke	1. Restarting incomplete fermentations: effect of acetic acid on different yeast strains 2. Off-flavours in wine from <i>Dekkera/Brettanomyces</i> yeast and lactic acid bacteria: survey of 4-ethylphenol in Australian wines 3. Off-flavours in wine from <i>Dekkera/Brettanomyces</i> and lactic acid bacteria	E&J Gallo Winery, Modesto, California, USA	17 May 2000
P.A. Henschke	1. Restarting incomplete fermentations: effect of acetic acid on different yeast strains 2. Off-flavours in wine from <i>Dekkera/Brettanomyces</i> yeast and lactic acid bacteria: survey of 4-ethylphenol in Australian wines 3. Off flavours in wine from <i>Dekkera/Brettanomyces</i> yeast and lactic acid bacteria: mousy N-heterocycles from <i>Dekkera/Brettanomyces</i> and lactic acid bacteria	Robert Mondavi, Windsor, California USA	18 May 2000
C.S. Stockley	Health benefits of wine	Dietician's Association of Australia 19th National Conference, Canberra ACT	18 May 2000
P.A. Henschke	1. Restarting incomplete fermentations: effect of acetic acid on different yeast strains 2. Off flavours in wine from <i>Dekkera/Brettanomyces</i> yeast and lactic acid bacteria: mousy N-heterocycles from <i>Dekkera/Brettanomyces</i> and lactic acid bacteria	Seminar sponsored by the Trellis Alliance of the University of California at Davis in cooperation with the UC Davis University Extension, Sonoma, California, USA	19 May 2000
P.B. Høj, E.J. Waters, D.B. Tattersall ⁸ , K. Adams ⁸ , K.F. Pocock, Y. Hayasaka, R. van Heeswijck ⁸	The haze proteins of grapes – PR proteins synthesised in a coordinate fashion following the onset of veraison	6th International Symposium on Grapevine Physiology and Biotechnology, Heraklion, Crete, Greece	11-16 June 2000
C.S. Stockley	Advances in Australian research into the potential cardioprotective effects of wine	XXVth World Congress of the Office International de la Vigne et du Vin, Paris, France	19 June 2000



Appendix 1
External seminars, talks and poster papers presented by
Institute staff during 1999/00

Author	Title	Organisation/Location	Date
P.B. Høj, E.J. Waters, D.B. Tattersall ⁸ , K. Adams ⁸ , K.F. Pocock, Y. Hayasaka, R. van Heeswijck ⁸	The haze proteins of wine – PR proteins synthesised in a coordinate fashion following the onset of veraison	50th Meeting, American Society of Enology and Viticulture, Seattle, Washington USA	19-23 June 2000
P.A. Henschke	1. New generation wine yeasts 2. Genetic strategies to reduce H ₂ S formation by yeast	Yeast Products and Discovery Conference, Couran Cove Resort, South Stradbroke Island, Qld	26 June – 1 July 2000
C.M. Sutherland, M.A. de Barros Lopes, P.R. Langridge ² , P.A. Henschke	A genetic strategy to reduce sulfite reductase activity in <i>Saccharomyces cerevisiae</i>	First Australian Conference on Yeast: products and discovery. Qld	30 June 2000
J. Bellon, A.J. Heinrich N. Shirley ⁸ , P. Ganter ¹² , S. Rainieri ¹ , P.R. Langridge ² , P.A. Henschke, V. Jiranek ⁸ , M.A. de Barros Lopes	Fluorescent AFLP analysis of genetic variation in <i>Saccharomyces</i> species and its application in wine biotechnology	First Australian Conference on Yeast: products and discovery. Qld	1 July 2000

Posters

Author	Title	Organisation/Location	Date
J.M. Eglinton	Complexing wine flavour with indigenous non- <i>Saccharomyces</i> wine yeasts	Fifth International Symposium on Cool Climate Viticulture and Oenology. Melbourne Vic	16-20 January 2000
J.M. Eglinton, M. Fogarty, S. McWilliam, I.L. Francis, M.J. Kwiatkowski, M.A. de Barros Lopes, S. Rainieri ¹ , P.B. Høj, P.A. Henschke	Flavour modification by selected use of <i>Saccharomyces bayanus</i>	Fifth International Symposium on Cool Climate Viticulture and Oenology. Melbourne Vic	16-20 January 2000
H. Gockowiak, P.A. Henschke	Effect of reactivation protocol and inoculation timing on the duration of malolactic fermentation	Fifth International Symposium on Cool Climate Viticulture and Oenology. Melbourne Vic	16-20 January 2000
Y. Hayasaka, E.J. Bartowsky	Development of a simple and accurate analytical method for diacetyl in wine using SPME and GC-MS	Fifth International Symposium on Cool Climate Viticulture and Oenology. Melbourne Vic	16-20 January 2000
A. Soden, I.L. Francis, H. Gockowiak, T.H. Lee ¹⁵ , P.A. Henschke	Complexing wine flavour with indigenous non- <i>Saccharomyces</i> wine yeasts	Fifth International Symposium on Cool Climate Viticulture and Oenology. Melbourne Vic	16-20 January 2000
J. Gardner ⁸ , M. Wenk ⁸ , M.A. de Barros Lopes, V. Jiranek ⁸	Identification of genes contributing to a ‘High Nitrogen Efficiency’ (HNE) phenotype in a modified wine yeast	Fifth International Symposium on Cool Climate Viticulture and Oenology. Melbourne Vic	16-20 January 2000
K. Poole ⁸ , M.A. de Barros Lopes, V. Jiranek ⁸	Growth and fermentation characteristics of yeast strains constitutively transporting proline	Fifth International Symposium on Cool Climate Viticulture and Oenology. Melbourne Vic	16-20 January 2000



Author	Title	Organisation/Location	Date
J. Bellon, M.A. de Barros Lopes	The analysis of genetic variation in <i>Saccharomyces</i> yeasts using AFLP	21st Annual Conference on the Organisation and Expression of the Genome. Lorne, Vic	13-17 February 2000
S.L. Brown, M.A. de Barros Lopes, P.B. Høj, E.J. Waters	Investigation into the biological role and mechanism of action of yeast mannoproteins which reduce visible haziness in wine	21st Annual Conference on the Organisation and Expression of the Genome. Lorne, Vic	13-17 February 2000
A.J. Heinrich, M.A. de Barros Lopes, J.M. Eglinton, A.-U.-Rehman ⁸ , H. Gockowiak, P.R. Langridge ²	Development of a low fermentation efficiency wine yeast	21st Annual Conference on the Organisation and Expression of the Genome. Lorne, Vic	13-17 February 2000
J. Gardner ⁸ , M. Wenk ⁸ , M.A. de Barros Lopes, V. Jiranek ⁸	Identification of genes contributing to a ‘High Nitrogen Efficiency’ (HNE) phenotype in a modified wine yeast	21st Annual Conference on the Organisation and Expression of the Genome. Lorne, Vic	13-17 February 2000

Advanced Wine Assessment Course and workshops

Author	Title	Organisation/Location	Date
P.W. Godden, M. Gishen, J.B. Hughes, A. Loveys, E. Robinson, A.D. Coulter, I.L. Francis	Advanced Wine Assessment Course	Waite Campus, Urrbrae SA	20-22 July 1999 7-10 December 1999
A.N. Sas	Workshop on use of ELISA kits for measuring pesticides in wine	Laboratory staff from six wineries, Berri Estates, Glossop, SA	14 July 1999
A.N. Sas	Chemical use, resistance and residues	Department of Natural Resource and Energy, Trawool, Vic	13 August 1999
M. Gishen	Quality management	Fifth International Cool Climate Symposium, Melbourne, Vic	17 January 2000
P.A. Henschke	Fermentation and spoilage	Fifth International Cool Climate Symposium, Melbourne, Vic	19 January 2000



Appendix 2
Teaching responsibilities of Institute staff during 1999/2000

Subject	No. of lectures	Institute staff
1999—Semester 2		
Adelaide University		
9685 Advances in Oenology	2	Dr I.L. Francis
	3	Dr P.A. Henschke
	2	Dr E.J. Bartowsky
	1	Dr P.J. Costello
9086 Advances in Oenology (Supplemented)	1	Dr E.J. Bartowsky
	1	Dr P.J. Costello
	1	Dr P.A. Henschke
1858 Wine packaging and quality management	1	C.S. Stockley
1958 Wine packaging and quality management	1	M. Gishen
5153 Viticulture B	3	A.N. Sas
International College of Hotel Management		
Wine Studies I	1	C.S. Stockley
The Flinders University of South Australia		
MMED 3921 Industrial and Pharmaceutical Microbiology	1	Dr P.A. Henschke
2000—Semester 1		
Adelaide University		
2580 Stabilisation and clarification	1	P.W. Godden
	3	Dr E.J. Waters
2582 Biotechnology	1	Dr M.A. de Barros Lopes
2213 Grape Industry Practice Policy and Communication (subject coordination and delivery for five weeks)	~40 hours	Professor P.B. Høj and C.S. Stockley
1005/3113 Winemaking	2	Dr P.A. Henschke
The University of South Australia		
BMgt (Wine Marketing)	1	C.S. Stockley
International College of Hotel Management		
Wine Studies I	1	C.S. Stockley
The University of New South Wales		
GENB 7001	1	C.S. Stockley



Appendix 3
Graduate and Honours student supervision responsibilities
of Institute staff for 1999/00

Student	Supervisor/s	Source of funds
Honours		
L. Brightman	I.L. Francis, P.B. Høj	Adelaide University
J. Campbell	A.P. Pollnitz, M.A. Sefton	Adelaide University
D. Dowden	E.J. Waters, G.P. Jones ⁸	Adelaide University
S. Franke	M.A. Sefton and H.P. McLean ⁸	Adelaide University
A. Green	M.A. de Barros Lopes	Adelaide University
H. McCarthy	E.J. Bartowsky, I.L. Francis	Adelaide University
M. Pearce	A.J. Markides ⁸ , E.J. Bartowsky	Adelaide University
J. Trotter	E.J. Waters, M.A. Sefton, P.B. Høj	Adelaide University
N. Viergutz	P.J. Costello, A.J. Markides ⁸	Adelaide University
Masters		
D. Coates	R. Gawel ⁸ , E.J. Bartowsky	Adelaide University
PhD		
R. Asenstorfer	G.P. Jones ⁸ , P.J. Iland ⁸ , E.J. Waters	GWRDC
S.L. Brown	M.A. de Barros Lopes, E.J. Waters, P.B. Høj	GWRDC
F. Carrau	P.A. Henschke, E. Dellacassa ¹³	University of the Republic of Uruguay staff member
N. D’Incecco	P.A. Henschke, E.J. Bartowsky	Italian PhD scholarship
J.M. Eglinton	P.A. Henschke, P.R. Langridge ²	Institute staff
D. Lee	G.P. Jones ⁸ , E.J. Waters	CRCV
J. Gardner	V. Jiranek ⁸ , M.A. de Barros Lopes	APA
R. Gawel	I.L. Francis, A.J. Markides ⁸	DHVO staff
W. Greenrod	M. Fenech ⁹ , M. Abbey ⁹ , P. Burcham ⁹ , C.S. Stockley	GWRDC
K. Howell	G.H. Fleet ¹⁰ , P.A. Henschke, E.J. Bartowsky	APRA scholarship
A. Oberholster	E.J. Waters, I.L. Francis, G.P. Jones ⁸ , P.G. Iland ⁸	GWRDC
A.P. Pollnitz	M.A. Sefton, G.P. Jones ⁸	Institute staff
K. Poole	V. Jiranek ⁸ , M.A. de Barros Lopes	APA
V.J. Stockdale	E.J. Waters, P.J. Williams, G.B. Fincher ²	
C. Smyl ¹⁴	P.A. Henschke, P.R. Langridge ²	CRCV/Commonwealth PG award
N.A. Yap	M.A. de Barros Lopes, P.A. Henschke, P.R. Langridge ²	GWRDC

Thesis completed

Student	PhD/Honours	Title	Supervisor/s
C.M. Sutherland	PhD	A genetic strategy to reduce sulfite reductase activity in <i>Saccharomyces cerevisiae</i>	M.A. de Barros Lopes, P.A. Henschke, P.R. Langridge ²
1	<i>Dipartimento di Protezione e Valorizzazione Agroalimentare, University of Bologna, Italy</i>	9	<i>CSIRO Health Sciences and Nutrition</i>
2	<i>Department of Plant Science, Adelaide University</i>	10	<i>Department of Food Science, University of New South Wales</i>
3	<i>BRL Hardy</i>	11	<i>Linear NIR Technology</i>
4	<i>Ridley Corporation</i>	12	<i>Tennessee State University</i>
5	<i>Southcorp Wines</i>	13	<i>University of the Republic of Uruguay</i>
6	<i>Agritechnology Pty Ltd</i>	14	<i>Funding ceased – student completing thesis</i>
7	<i>Orlando Wyndham</i>	15	<i>E+J Gallo Winery</i>
8	<i>Department of Horticulture, Viticulture and Oenology, Adelaide University</i>		



Appendix 4

Institute staff publications 1999/00

- 596 Weeks, S.; Sefton, M. Analysis of TCA in wines and oakwood. *Aust. N.Z. Wine Ind. J.* 14 (1): 56; 1999.
- 597 Eglinton, J.M.; Henschke, P.A. The occurrence of volatile acidity in Australian wines. *Aust. Grapegrower Winemaker*. (426a): 7-12; 1999.
- 598 Bartowsky, E.J.; Henschke, P.A. Use of a polymerase chain reaction for specific detection of the malolactic fermentation bacterium *Oenococcus oeni* (formerly *Leuconostoc oenos*) in grape juice and wine samples. *Aust. J. Grape Wine Res.* 5: 39-44; 1999.
- 599 Eglinton, J.M.; Henschke, P.A. Restarting incomplete fermentations: the effect of high concentrations of acetic acid. *Aust. J. Grape Wine Res.* 5: 71-78; 1999.
- 600 Gray, J.D.; Kolesik, P.; Høj, P.B.; Coombe, B.G. Confocal measurement of the three-dimensional size and shape of plant parenchyma cells in a developing fruit tissue. *Plant J.* 19: 229-236; 1999.
- 601 Kotseridis, Y.; Baumes, R.L.; Bertrand, A.; Skouroumounis, G.K. Quantitative determination of 2-methoxy-3-isobutylpyrazine in red wines and grapes of Bordeaux using a stable isotope dilution assay. *J. Chromatogr. A* 841: 229-237; 1999.
- 602 Wirthensohn, M.G.; Francis, I.L.; Gawel, R.; Jones, G.P. Sensory-instrumental correlation of extra virgin olive oil aromas using a 'chemical nose'. Iles, P.; Knox, E., eds. *Proceedings of the Australian international symposium on analytical science: incorporating 15AC and Chromatography '99*; 4-9 July 1999. North Melbourne, Vic: Royal Australian Chemical Institute; 1999: 75-78.
- 603 Sas, A. Residue management in the wine industry. Rowland, P.; Bradford, D., eds. *Proceedings of the Australian national pesticide risk reduction workshop*; 16-18 April 1997. Canberra, ACT: Bureau of Rural Sciences, Agriculture, Fisheries and Forestry; 1998: 155-158.
- 604 Stockley, C.S. An explanation of recent wine and health issues. *Aust. Grapegrower Winemaker* (429): 15-21; 1999.
- 605 Pollnitz, A.P.; Jones, G.P.; Sefton, M.A. Determination of oak lactones in barrel-aged wines and in oak extracts by stable isotope dilution analysis. *J. Chromatogr. A* 857: 239-246; 1999.
- 606 Høj, P.B.; Hayes, P.F. The Australian wine industry's research and development effort and its importance for sustained growth. Blair, R.J.; Sas, A.N.; Hayes, P.F.; Høj, P.B., eds. *Proceedings of the tenth Australian wine industry technical conference*; 2-5 August 1998; Adelaide, SA: Australian Wine Industry Technical Conference Inc.; 1999: 10-15.
- 607 Francis, I.L.; Iland, P.G.; Cynkar, W.U.; Kwiatkowski, M.; Williams, P.J.; Armstrong, H.; Botting, D.G.; Gawel, R.; Ryan, C. Assessing wine quality with the G-G assay. Blair, R.J.; Sas, A.N.; Hayes, P.F.; Høj, P.B., eds. *Proceedings of the tenth Australian wine industry technical conference*; 2-5 August 1998; Adelaide, SA: Australian Wine Industry Technical Conference Inc.; 1999: 104-108.
- 608 Jones, G.P.; Asenstorfer, R.E.; Iland, P.G.; Waters, E.J. Colour, phenolics and tannins in wines. Blair, R.J.; Sas, A.N.; Hayes, P.F.; Høj, P.B., eds. *Proceedings of the tenth Australian wine industry technical conference*; 2-5 August 1998; Adelaide, SA: Australian Wine Industry Technical Conference Inc.; 1999: 109-112.
- 609 Sas, A.N.; Stevens, R.M. Meeting a salt specification. Blair, R.J.; Sas, A.N.; Hayes, P.F.; Høj, P.B., eds. *Proceedings of the tenth Australian wine industry technical conference*; 2-5 August 1998; Adelaide, SA: Australian Wine Industry Technical Conference Inc.; 1999: 116-120.
- 610 Godden, P.W.; Pollnitz, A.P.; Osicka, S.; Sykes, M.; Liacopoulos, D.; Pardon, K.H.; Spillman, P.J.; Gawel, R.; Jones, G.P.; Skouroumounis, G.K.; Sefton, M.A. Measuring desirable oakwood components in wine. Blair, R.J.; Sas, A.N.; Hayes, P.F.; Høj, P.B., eds. *Proceedings of the tenth Australian wine industry technical conference*; 2-5 August 1998; Adelaide, SA: Australian Wine Industry Technical Conference Inc.; 1999: 121-124.
- 611 van Heeswijck, R.; Høj, P.B. Overview of gene technology. Blair, R.J.; Sas, A.N.; Hayes, P.F.; Høj, P.B., eds. *Proceedings of the tenth Australian wine industry technical conference*; 2-5 August 1998; Adelaide, SA: Australian Wine Industry Technical Conference Inc.; 1999: 125-128.
- 612 Robinson, S.P.; Thomas, M.; Scott, N.S.; Dry, I.; Davies, C.; Franks, T.; Boss, P.; Høj, P.B.; van Heeswijck, R. Application of gene technology in viticulture. Blair, R.J.; Sas, A.N.; Hayes, P.F.; Høj, P.B., eds. *Proceedings of the tenth Australian wine industry technical conference*; 2-5 August 1998; Adelaide, SA: Australian Wine Industry Technical Conference Inc.; 1999: 134-138.
- 613 Skerrett, J.H.; Phongkham, T.; Sas, A.N. New approaches for residue detection by the Australian wine industry. Blair, R.J.; Sas, A.N.; Hayes, P.F.; Høj, P.B., eds. *Proceedings of the tenth Australian wine industry technical conference*; 2-5 August 1998; Adelaide, SA: Australian Wine Industry Technical Conference Inc.; 1999: 139-145.
- 614 Soden, A.; Francis, I.L.; Gockowiak, H.; Henschke, P.A. The use of non-*Saccharomyces* yeasts in winemaking. Blair, R.J.; Sas, A.N.; Hayes, P.F.; Høj, P.B., eds. *Proceedings of the tenth Australian wine industry technical conference*; 2-5 August 1998; Adelaide, SA: Australian Wine Industry Technical Conference Inc.; 1999: 166-171.
- 615 Liacopoulos, D.; Barker, D.; Howland, P.R.; Alcorso, D.C.; Pollnitz, A.P.; Skouroumounis, G.K.; Pardon, K.H.; McLean, H.J.; Gawel, R.; Sefton, M.A. Chloroanisole taint in wines. Blair, R.J.; Sas, A.N.; Hayes, P.F.; Høj, P.B., eds. *Proceedings of the tenth Australian wine industry technical conference*; 2-5 August 1998; Adelaide, SA: Australian Wine Industry Technical Conference Inc.; 1999: 224-226.
- 616 Capone, D.L.; Skouroumounis, G.K.; Barker, D.A.; McLean, H.J.; Pollnitz, A.P.; Sefton, M.A. Absorption of chloroanisoles from wine by corks and by other materials. *Aust. J. Grape Wine Res.* 5: 91-98; 1999.
- 617 Jones, P.R.; Møller, B.L.; Høj, P.B. The UDP-glucose:p-hydroxymandelonitrile-O-glucosyltransferase that catalyzes the last step in synthesis of the cyanogenic glucoside dhurrin in *Sorghum bicolor*: isolation, cloning, heterologous expression, and substrate specificity. *J. Biol. Chem.* 274: 35483-35491; 1999.
- 618 Høj, P.; Keniry, J.; Day, R.; Gerlach, S. The Australian Wine Industry: success through innovation: a paper prepared by an independent working group for consideration by the Prime Minister's Science, Engineering and Innovation Council at its fourth meeting; 26 November 1999: Canberra. ACT: Department of Industry, Science and Resources; 1999: 34p. (occasional paper no. 3).
- 619 Costello, P.J.; Lee, T.H.; Henschke, P.A. Mousy off-flavour spoilage of wine by lactic acid bacteria. Lonvaud-Funel, A., ed. *Œnologie 99: 6e Symposium international d'œnologie*; Paris: Editions TEC & DOC; 2000: 226-230.
- 620 Waters, E.J.; Høj, P. Grape proteins involved in white wine instability: a short review of current knowledge and future perspectives. Lallemand, S.A. *Colloids and mouthfeel in wines*. Toulouse Cedex, France: Lallemand; 1999: 41-49.
- 621 Blair, R. Information services from The Australian Wine Research Institute. *Aust. N.Z. Wine Ind. J.* 15: 95-97; 2000.
- 622 Gishen, M. Integration of HACCP into quality management systems in the wine industry - From Grapes to Glass. Peters, D., ed. *Proceedings of the 6th Australian HACCP conference*; 10-12 August 1999; Oatlands, NSW: Food Operations; 1999: 92-94.
- 623 Pollnitz, A.P.; Pardon, K.H.; Sefton, M.A. Quantitative analysis of 4-ethylphenol and 4-ethylguaiaicol in red wine. *J. Chromatogr. A* 874: 101-109; 2000.
- 624 Dupin, I.V.S.; Stockdale, V.J.; Williams, P.J.; Jones, G.P.; Markides, A.J.; Waters, E.J. *Saccharomyces cerevisiae* mannoproteins that protect wine from protein haze: evaluation of extraction methods and immunolocalization. *J. Agric. Food Chem.* 48: 1086-1095; 2000.
- 625 Soden, A.; Francis, I.L.; Oakey, H.; Henschke, P.A. Effects of co-fermentation with *Candida stellata* and *Saccharomyces cerevisiae* on the aroma and composition of Chardonnay wine. *Aust. J. Grape Wine Res.* 6:21-30; 2000.
- 626 Damberg, R.G.; Kambouris, B.; Gishen, M.; Francis, I.L. Measuring fruit quality. Davies, C.; Dundon, C.; Hamilton, R., eds. *Modern viticulture - meeting market specifications: proceedings of a seminar*; 15 July 1999 at the Barossa Convention Centre, Tanunda; 25 August 1999 at the Cowra Civic Centre, Cowra; 3 September 1999 at the Mildura Arts Centre, Mildura; and 16 September at the Technology Park Function Centre, Perth. Adelaide, S.A.: Australian Society of Viticulture and Oenology; 2000: 45-47.
- 627 Pollnitz, A.P.; Pardon, K.H.; Sefton, M.A. 4-ethylphenol and 4-ethylguaiaicol in Australian red wines. Allen, M.; Wall, G., eds. *Use of oak barrels in winemaking: proceedings of a seminar*; 15 July 1999 at the Barossa Convention Centre, Tanunda; 25 August 1999 at the Cowra Civic Centre, Cowra; 3 September 1999 at the Mildura Arts Centre, Mildura; and 16 September at the Technology Park Function Centre, Perth. 7 Oct 1999, Adelaide, S.A.: Australian Society of Viticulture and Oenology; 2000: 10-11.
- 628 Pocock, K.F.; Hayasaka, Y.; McCarthy, M.G.; Waters, E.J. Thaumatin-like proteins and chitinases, the haze forming proteins of wine, accumulate during the ripening of grape (*Vitis vinifera*) berries and drought stress does not affect the final levels per berry at maturity. *J. Agric. Food Chem.* 48:1637-1643; 2000.



Appendix 5
Institute Committees

Staff Member	Management Advisory	Research Steering	Industry Services Steering	Communication Steering
Peter Høj	C	C	X	X
Eveline Bartowsky				
Sally-Jean Bell	X	X	X	
Rae Blair	X			C
Don Buick	X		X	
Adrian Coulter	X	X	X	
Janet Currie	X			
Catherine Daniel				X
Miguel de Barros Lopes	X	X		
Jeff Eglinton				X
Leigh Francis	X	X	X	
Mark Gishen	X	X	X	
Holger Gockowiak			X	
Peter Godden	X	X	C	X
Jeremy Hack				
Yoji Hayasaka	X	X	X	
Paul Henschke	X	X		
Matthew Holdstock				
John Hughes				
Ingrid Oats				
Anita Oberholster				
Ken Pocock				
Alan Pollnitz				
Mark Sefton	X	X		
Creina Stockley		X		X
Elizabeth Waters	X	X		



Analytical Service Steering	Information Technology	Biosafety	Occupational Health and Safety	Superannuation	Staff Code Negotiation
X	X				X
X		X		X	
X					
X					
X					
			X	X	
	X				
C	C				
			X		
C					X
	X				
X					
X			C		
	X				
		X			
				X	
		X			
	X	X	C	X	
X					
	X				



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